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Abortive Meiosis in Plasmodial Pollen Mother Cells of *Helianthemum*

BY

BRIAN SNOAD

(*John Innes Horticultural Institution, Bayfordbury, Hertford, Herts.*)

With Plate 1 and two Figures in the Text

Abstract

The anthers of two garden varieties of *Helianthemum* contain not only normal pollen mother cells but also a large proportion of plasmodia with varying numbers of nuclei of varying nuclear complements. These abnormal cells probably resulted from a split spindle associated with a failure of wall formation during several premeiotic mitoses. Chromosome pairing at meiosis is higher than might be expected and in abnormal tissues such as these it provides evidence for 'somatic pairing' having taken place during the premeiotic mitoses. Meiosis stops at the end of the first division in the plasmodia and although it is completed in the normal pollen mother cells viable pollen is never produced.

1. Introduction

THE breakdown of meiosis can generally be attributed to one of two main types of abnormality which are not uncommon in their occurrence. Firstly, owing to structural, genotypic, and environmental influence—particularly in hybrids—there may be a reduction of chromosome pairing and even complete asynapsis. Secondly, spindle abnormalities may occur which affect the movement of the chromosomes during first and second division and also hinder the formation of cell walls.

In *Helianthemum* the breakdown of meiosis is probably caused by a less common spindle abnormality preventing wall formation in parts of the sporogenous tissue during several premeiotic mitoses.

Six garden varieties of *Helianthemum* growing at the John Innes Horticultural Institution were examined.

	$2x = 20$	$3x = 30$
Normal	*'Huntsman'*	*'Golden Queen'*
	'Taylor's Seedling'	*'Salmon Queen'*
Abnormal	*'Long Sutton'*	. .
	'Mrs. Smith'	. .

Helianthemum vulgare exists in many forms and colours and it is assumed (Robinson, 1900) that it is mainly responsible for the majority of garden forms in existence today.

[Annals of Botany, N.S. Vol. XVIII, No. 69, January, 1954.]

2. METHODS

Anthers were fixed in acetic alcohol (1:3) for 15 minutes and then hardened overnight in 95 per cent. alcohol. Squash preparations were made using Feulgen staining (Darlington and La Cour, 1947). In order to stain the cytoplasm and thus render the cell boundaries more distinct the preparations were passed through a solution of Ponceau P.R. in 80 per cent. alcohol before being made permanent. The cytoplasm was stained a pale pink.

In addition other anthers were prepared for sectioning by the paraffin method using La Cour's 2BX (Darlington and La Cour, 1947) as a fixative. They were cut at 18 μ and stained with crystal violet.

The observations are largely based upon the study of the two abnormal diploids.

3. NORMAL MEIOSIS

In the normal diploid varieties ten bivalents are regularly formed with one or two chiasmata per bivalent. Anaphase separation is normal and viable pollen is formed. In the varieties showing the abnormality a small and variable percentage of cells undergoes a normal meiosis too, but without, as we shall see, giving rise to viable pollen.

The two triploid varieties, which probably arose after the non-reduction of a gamete, show the formation of up to four trivalents at meiosis and a consequent irregular chromosome distribution.

4. THE BREAKDOWN OF MEIOSIS

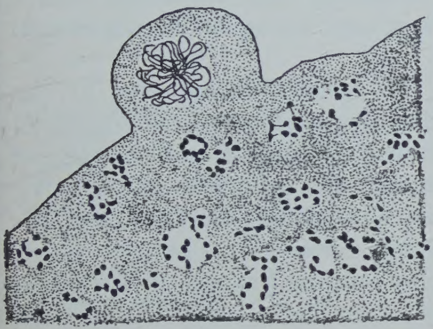
Masses of cytoplasm with no cell walls and containing many nuclei are to be seen within the anthers of the two abnormal varieties (Pl. 1, Fig. 1). These plasmodia regularly occur in all the anthers in association with a variable proportion of normal pollen mother cells.

The sectioned material showed that the plasmodia are distributed at random within the anthers and also that the tapetum appeared to be normally developed. The plasmodia vary greatly in size and contain from 2 to approximately 80 nuclei which are not limited to a doubling series 2, 4, 8, 16, &c.; odd numbers of nuclei often occur in complete masses of cytoplasm because of the probable multipolar nature of the spindle. Many nuclei are incomplete, and may contain anything from 4 to 20 chromosomes. Most chromosomes are paired during meiosis.

The nuclei, which have normal nuclear membranes, are contained in an apparently uninterrupted cytoplasm without any detectable peripheral wall (Pl. 1, Fig. 2). Typically the plasmodia have a smooth and regular outline, deviation only occurring where the contour is broken by the presence of a nucleus greatly out of phase with the remaining mass (Text-fig. 1). This would indicate the presence of a partially separating membrane between the cytoplasm containing the nucleus with the divergent behaviour and the main body of the plasmodium. A thin peripheral membrane around the individual plasmodia must also be suspected.

In all the plasmodia examined all the nuclei are apparently capable of beginning meiosis. Pairing of the chromosomes is higher than might be expected in the deficient nuclei and those with only four chromosomes often contain two bivalents. First anaphase separation is normal, the spindles being orientated at random within each plasmodium. With the completion of first telophase meiosis proceeds no further.

Within a typical plasmodium the divisions are almost perfectly synchronized and this synchronization is independent of the number of chromosomes in the nuclei (Pl. 1, Fig. 4). Greater differences in the stage of division are seen in those plasmodia containing a partially included nucleus and its accompanying cytoplasm on the periphery. Even wider differences occur between different plasmodia within an anther. Generally the normal pollen mother cells are further advanced than the plasmodia.



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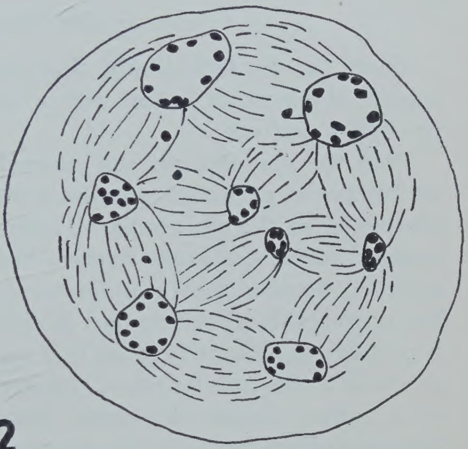

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Text-fig. 1. Portion of plasmodium showing partially included cytoplasm and nucleus at pachytene and the other nuclei at interphase. (× 700.)

Text-fig. 2. Small plasmodium with interphase nuclei with different numbers of chromosomes and persisting primary and secondary spindles. (× 1,400.)

One plant of '*Mrs Smith*' sported giving rise to flowers with yellow segments on otherwise normal red petals—a not uncommon occurrence in this variety. The younger flower-buds of this sport were examined, but there is little difference from the ordinary form. There is still a mixture of normal pollen mother cells and plasmodia. At meiosis in the normal pollen mother cells the chromosome number is regular and the only difference lies in the slight failure of pairing and the consequent irregular distribution of the chromosomes at first anaphase.

5. Pollen Sterility

Meiosis in the plasmodia never proceeds beyond the first division, although there is an indication that in some cases the cytoplasm may continue to develop physiologically. It becomes more granular and coarse and resembles the condition seen in the formation of normal pollen grains. Possibly this continuation in development is related to the positions of the plasmodia with regard to the tapetum. In time they begin to dry up and so degenerate. Likewise, the potential pollen grains which arise from the normal pollen mother cells in the presence of the plasmodia also seem incapable of complete development. This is no doubt due to the presence of the plasmodia. No viable pollen therefore occurs in these abnormal plants.

6. Origin and Comparative Behaviour

There can be little doubt that these plasmodia have originated from a spindle abnormality linked with a failure of wall formation during several premeiotic mitoses.

That the spindles are not normal in this material is indicated by the appearance of persistent spindles during the interphase of meiosis in some of the nuclei in the plasmodia. These were mainly evident in the sectioned material where the osmic fixation had rendered the cytoplasm and spindles obvious. Some of these spindles were between unrelated nuclei and therefore had presumably played no part in chromosome movement. This observation is perhaps significant because it is paralleled by what happens in *Iris* endosperm at a non-cellular phase (Jungers, 1931) and in the pollen mother cells of a *Festuca-Lolium* derivative (Darlington and Thomas, 1937). In the *Festuca-Lolium* derivative the abnormal spindles—both primary and secondary—are not only associated with irregular chromosome distribution but also with the failure of wall formation. This shows that the spindle plays an important part in the formation of the cell wall.

The varying numbers of chromosomes in the nuclei of the plasmodia could have been caused by their random distribution after a complete suppression of the spindle. The absence of multivalent associations and of nuclei with more than twenty chromosomes excludes this possibility. A far more likely assumption is that the spindle was split and this resulted in nuclei with variable chromosome complements. In this case the spindle will have functioned for chromosome movement and the daughter chromatids will have been separated. Multivalent associations will not then be formed unless restitution of the parent nuclei occurs. These observations are confirmed by the non-appearance of multivalents in the material examined.

The observations made upon meiosis also throw light upon the possible internal nuclear arrangements of the premeiotic cells in the anthers. In the plasmodia there was an obviously higher frequency of chromosome pairing than might be expected after the nuclear complements had been disrupted by irregular spindle behaviour. This high frequency could have arisen as a result of 'somatic pairing' during previous (premeiotic) mitoses. Such a habit would obviously greatly facilitate pairing of the chromosomes at meiosis. In aberrant cells such as these it is easier to obtain evidence for suggesting that homologous chromosomes had been lying close together during earlier mitoses.

TABLE I

Comparison of Behaviour in Several Genera containing Plasmodial Sporogenous Tissue

Determination	*Calamagrostis* (Nygren, 1946) Apomictic clone	*Zea* (Beadle, 1932) Recessive gene	*Zea* (Lebedeff, 1940) Induced mutation	*Hordeum* (L. Smith, 1942) Recessive gene	*Helianthemum* Vegetative clone
High frequency of abnormal tissues	−	−	+	+	+
Formed during as well as before meiosis	+	−	+	+	−
All plasmodia active	−	+	+	+	+
Chromosome number variability in plasmodia	−	−	−	+	+
Nuclear fusion at MI	−	+	−	+	−
Restitution at TI	+	−	−	−	−
Meiosis completed	+	?	+	+	−
Some viable pollen produced	+	+	−	?	−

When the abnormalities found in *Helianthemum* are compared with those in other genera (Table I) very few common characteristics are seen. The plasmodia reported in *Zea mays* (Lebedeff, 1940) occurred in a plant resulting from selfing for four generations plants grown from heat-treated seed. It is inferred that in *Calamagrostis* (Nygren, 1946) the occurrence of plasmodia may have been the result of extreme environmental conditions because several plants growing in the alpine regions of the Swedish mountains were all abnormal and produced no pollen. Whatever the reason for the original upset the formation of plasmodia is now under genotypic control. In *Helianthemum* natural variations in climatic conditions did not induce the formation of plasmodia in normal plants growing side by side with the abnormal ones.

The loss of the sexual process is not a great disadvantage in the garden forms of *Helianthemum* because they are usually easily propagated by vegetative means. It is worthy of note that neither of the sports that arose continued its vegetative growth and so could not be propagated.

SUMMARY

1. A mixture of normal pollen mother cells and plasmodia with nuclei of varying chromosome numbers occurs in differing proportions in all anthers of two garden varieties of *Helianthemum*.

2. Plasmodia are randomly distributed in otherwise normally developed anthers.

3. The plasmodia are probably caused by a split spindle linked with a failure of wall formation during premeiotic mitosis. Meiosis is normal in the plasmodia until it stops at the end of first division. Viable pollen is never produced even by the normal pollen mother cells.

4. Because of somatic pairing during premeiotic mitosis this abnormality results in there being a larger proportion of homologues per cell at meiosis than a random splitting would require.

ACKNOWLEDGEMENTS

I wish to thank Dr. C. D. Darlington and Mr. L. F. La Cour for their helpful criticism and advice.

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EXPLANATION OF PLATE I

Illustrating B. Snoad's paper on 'Abortive Meiosis in Plasmodial Pollen Mother Cells of *Helianthemum*'.

Plasmodia in two garden varieties of *Helianthemum*. Fixation in acetic-alcohol. Feulgen staining and counter staining with Ponceau P.R.

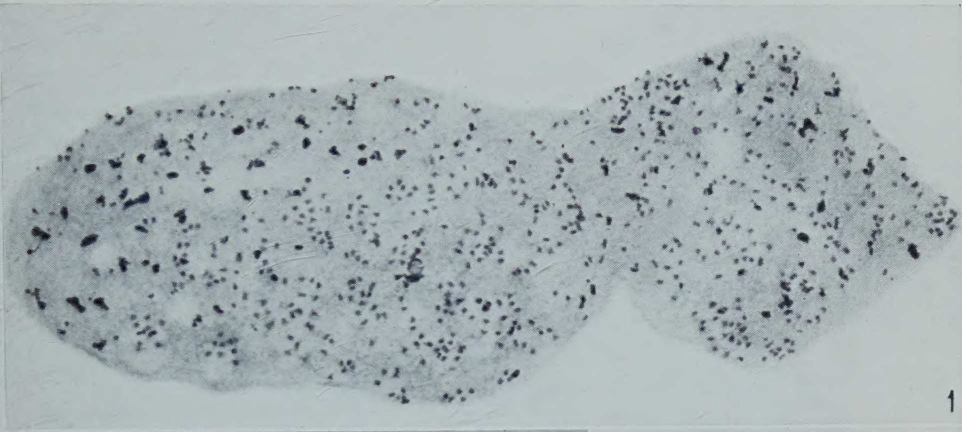
FIG. 1. Large plasmodium with many nuclei at interphase ('*Mrs. Smith*'). (× 360.)

FIG. 2. Small plasmodium with four nuclei at pachytene ('*Long Sutton*'). (× 1,000.)

FIG. 3. Metaphase nuclei in plasmodium with univalents off the spindle ('*Long Sutton*'). (× 1,000.)

FIG. 4. Portion of large plasmodium showing nuclei of varying size at diakinesis ('*Mrs. Smith*'). (× 1,000.)

FIG. 5. Diakinesis in a binucleate plasmodium ('*Mrs. Smith*'). (× 1,100.)



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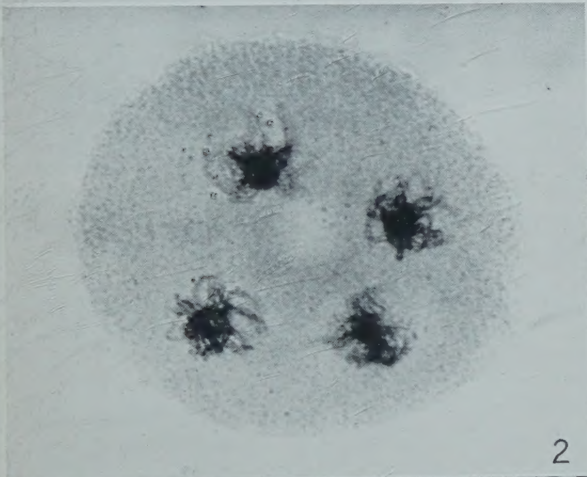

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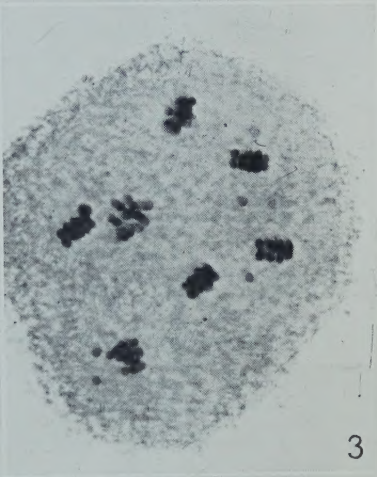

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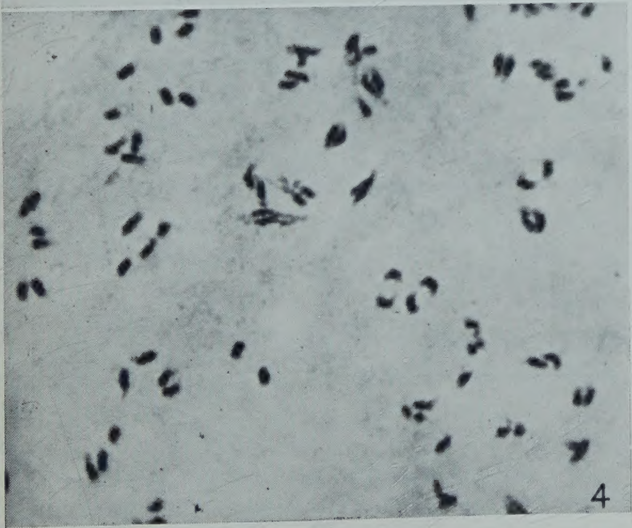

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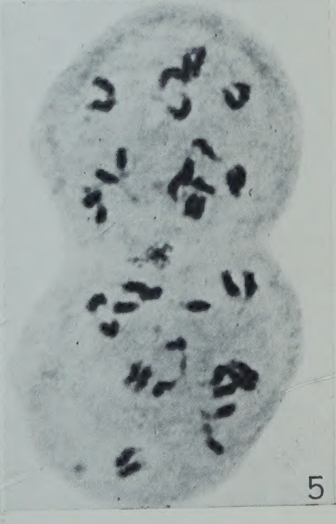

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B. SNOAD

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Spontaneous Chromosome Breakage in *Lilium* Endosperm

BY

R. D. BROCK

(*John Innes Horticultural Institution, Bayfordbury, Hertford, Herts.*)

With Plate 2 and one Figure in the Text

ABSTRACT

Abnormal mitoses, resulting from chromosome breakage and reunion, occurred in *Lilium regale* and in the hybrid *L.* '*Phyllis Cox*' 4 weeks after fertilization. The abnormalities appeared to commence with a single chromatid bridge and in '*Phyllis Cox*' abnormalities accumulated resulting in the formation of multiple bridges, ring chromosomes, fragments, dicentric chromosomes, and micronuclei. Failure of anaphase separation followed by restitution resulted in the formation of highly polyploid nuclei in which mass spontaneous chromosome breakage occurred.

The accumulation of abnormalities in the hybrid endosperm reduced the mitotic activity and culminated in the failure of the endosperm. This led to the death of the otherwise normal embryo. This sequence of events is probably of wide importance in the failure of interspecific hybridization.

1. INTRODUCTION

SPONTANEOUS chromosome breakage has been reported in endosperm tissue in *Zea mays* (Clark and Copeland, 1940), also in the tapetum of *Lilium canadense* (Cooper, 1933) and in pollen grains (Darlington and Upcott, 1941). Spontaneous changes in chromosomes occur under three general types of conditions: extreme natural conditions, special genotypic conditions, and differentiation within the organism. The spontaneous chromosome changes in endosperms fall into the last two categories, the tissue being polyploid and 'limited' in the respect that it is short-lived and has a special function. In this way the endosperm resembles the tapetum and the pollen grains.

The role of the endosperm in seed development has been reviewed by Brink and Cooper (1947). They consider the failure of the fertilized ovule to develop into a mature seed is frequently not a function of the embryo but a result of the abnormal development in the endosperm.

These studies are an attempt to elucidate this problem.

2. MATERIAL AND METHOD

The endosperms examined were collected from open pollinated flowers of *L. centifolium*, *L. regale*, and two hybrids growing in the grounds of this Institution. The hybrids were '*Phyllis Cox*' and '*Lilian Cummings*', sister seedlings from *L. davidi* var. *Willmottiae* crossed by *L. dauricum*. They are stated to set seed freely (Preston, 1939). In my experience, however, *L. regale* and

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L. centifolium set seed freely, but '*Phyllis Cox*' and '*Lilian Cummings*' set very few seeds (Table I).

TABLE I

Development of Ovules in Lilium Species and Hybrids. Ovule Development, Endosperm and Embryo Examination made 4 Weeks after Fertilization. Mature Seed Recorded 3 Months after Fertilization

Species or hybrids.	Ovules		No. of embryos and endo-sperms examined.	No. of abnormal endo-sperms.	No. of abnormal embryos.	Mature Seed (% of total ovules).
	Total.	Developing.				
L. centifolium	242	88%	6	0	0	81%
L. regale	1,172	78%	30	3	0	68%
'*Lilian Cummings*'	270	0·4%	1	0	0	0·4%
'*Phyllis Cox*'	3,291	0·7%	24	23	2	0·1%

Examination of pollen mother cells of these *Lilium* species and hybrids revealed surprisingly regular meiosis. Twelve bivalents were found in both species and hybrids and anaphase separations were regular, resulting in tetrads each with twelve chromosomes. Pollen was plentiful in both species and hybrids. Swelling of the seed pod was taken as an indication of fertilization and ovule development.

The morphology of the chromosomes of the developing embryos from open pollination of '*Phyllis Cox*' indicates that the embryos resulted from selfing and they will thus be F2.

Fixations were made 2 weeks and 4 weeks after fertilization, using the technique of Rutishauser and Hunzkier (1950). Ovules were removed from the seed pod, fixed in acetic alcohol (1:3) for 1 hour, then stored overnight in 80 per cent. alcohol. After successive transfers through 40 per cent. alcohol, 20 per cent. alcohol, and water, 15 minutes in each, ovules were hydrolysed in N/I HCl at 60° C. for 10 minutes and stained in Feulgen reagent for 2 hours. Endosperm and embryo were dissected, squashed in 45 per cent. acetic acid and slides made permanent by mounting in Euparal (Darlington and La Cour, 1947).

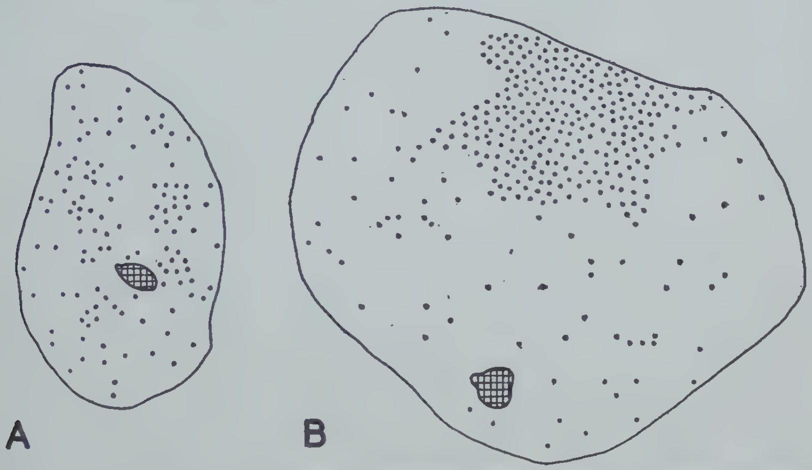
3. NORMAL DEVELOPMENT OF EMBRYO AND ENDOSPERM

In *Lilium* there is a diploid embryo ($2n = 24$) and a pentaploid endosperm ($5n = 60$) (Maheshwari, 1950). The embryo grows slowly at first and later more rapidly. Endosperm grows rapidly for 3 to 5 weeks. During this period it is non-cellular. Mitoses are at first synchronized and later occur in waves. Finally, at or about the time of cell wall formation mitoses occur at random and less frequently. The endosperm grows for a further 4 to 6 weeks before degenerating and being absorbed by the rapidly developing embryo.

4. OBSERVATIONS

Two weeks after fertilization very few ovules were developing in the hybrids and no mitoses were seen in the endosperms. Three nuclei had single chromatid bridges at anaphase in endosperms of *L. regale*. There were no abnormalities in embryos of either species or hybrids.

Four weeks after fertilization abnormal mitoses occurred in the endosperms of *L. regale* and '*Phyllis Cox*'. Two embryos of '*Phyllis Cox*' had no mitoses and their nuclei were degenerating. Both of these embryos were associated with endosperms which had completely degenerated.



TEXT-FIG. 1. Endosperms and embryos of '*Phyllis Cox*', 4 weeks old, showing difference in distribution of chromosome breakage and its effect on the rate of growth of endosperm and embryo. Chromosome breakage represented by dots. Embryos cross-hatched. A. Initial breakage early, distribution uniform, growth slow. B. Initial breakage later, distribution localized, growth more rapid. (× 8.)

All of the abnormal mitoses in the endosperms resulted from chromosome breakage and reunion and there was a correlation between the extent and distribution of chromosome breakage and the size of both endosperm and embryo. Endosperms with uniformly distributed chromosome breakage (Text-fig. 1A), indicative of abnormality first occurring at an early stage of development, were small in size. Endosperms with localized areas of chromosome breakage (Text-fig. 1B), indicative of the initial abnormality occurring later, were larger. Endosperms with no chromosome breakage were larger still. The size of the developing embryos paralleled that of their respective endosperms.

5. CHROMOSOME BREAKAGE AND REUNION

It was not possible to analyse sufficient metaphases so the analysis of the chromosome breakage and reunion was made at anaphase. Table II lists the frequency of various chromosome configurations in two endosperms of *L. regale* and ten of '*Phyllis Cox*'.

Bridges

The general pattern of development of the abnormalities appeared to commence with a single chromatid bridge (Pl. 2, Fig. 1). Mechanical breakage of

TABLE II

Anaphase Analysis of Chromosome Breakage and Reunion in L. regale *and* 'Phyllis Cox' *Endosperms 4 Weeks after Fertilization*

Endosperm.	Anaphases Total.	Broken.	Sister reunion bridges.	Acentric fragments.	Minute fragments.	Ring chromosomes.	Dicentric chromosomes.	B''/Total anaphases.
L. Regale								
1	7	3	1	3	—	—	—	*0·57*
2	42	3	3	1	—	—	—	*0·07*
Total *L. regale*	**49**	**6**	**4**	**4**	—	—	—	—
'Phyllis Cox'								
1	15	11	7	1	—	61	5	*8·33*
2	11	11	16	19	—	22	7	*7·45*
3	2	1	—	—	—	3	—	*3·0*
4	34	21	36	16	5	—	11	*2·0*
5	37	17	14	20	2	2	8	*1·43*
6	19	10	16	2	2	—	4	*1·25*
7	14	5	2	3	—	6	1	*1·15*
8	7	5	3	4	—	1	—	*1·14*
9	2	1	—	—	—	—	1	*1·0*
10	12	5	—	—	—	3	2	*0·83*
Total *'Phyllis Cox'*	**153**	**87**	**94**	**65**	**9**	**98**	**39**	—

this bridge at anaphase or telophase followed by sister reunion results in the breakage-fusion-bridge cycle described by McClintock (1941) and the bridge persists. *L. regale* showed only single bridges, but in '*Phyllis Cox*' there were anaphases with 1, 2, 3, 4, 5, 7, and 9 chromatid bridges.

Breakage of these bridges, and possibly further spontaneous chromosome breakage, followed by chromosome reunion results in the formation of ring chromosomes, long centric and acentric fragments, and dicentric chromosomes. As no cell walls are formed many of the bridges fail to break. Restitution can occur at the subsequent mitosis and highly polyploid nuclei result (Pl. 2, Fig. 5). Spontaneous chromosome breakage intensifies in highly polyploid nuclei resulting in mass chromosome breakage (Pl. 2, Fig. 7). These nuclei are the only ones which have breakage and reunion of chromatids as well as chromosomes. All other abnormal mitoses had only chromosome breakage followed by sister reunion or chromosome reunion.

Rings

In '*Phyllis Cox*' there were 98 ring chromosomes, 85 of which were acentric and 13 centric. Of the total, 80 per cent. were showing free separation and only 12 per cent. interlocking and 8 per cent. 'figure of eight' separation. However, it follows from the absence of free fragments that most of the nuclei had passed through at least one mitosis since the formation of the ring. The freely separating rings had average lengths of 11·4 μ for each ring, the 'figures of eight' had average total length of 25·9 μ, and the interlocked rings had average lengths of 19·5 μ for each ring.

Fragments

In highly polyploid nuclei of '*Phyllis Cox*' there were large numbers of small fragments and also fragments up to 67 μ long, the longest chromosome in the normal complement was 30 μ. This indicates that many of the fragments were included in the telophase nuclei and duplicated at subsequent divisions. In several nuclei the total of all the fragments indicates that only one chromosome fragmented. This may have occurred as a result of one chromosome being left out of the telophase nucleus, the increased unbalance at the subsequent division resulted in the chromosome from the micronucleus fragmenting.

Dicentric Chromosomes

The anaphase separation of 39 dicentric chromosomes was studied in '*Phyllis Cox*'; only 3 had parallel separation, 28 had criss-cross separation, 4 were interlocked, and 4 were interlocked with a loop. All of the dicentric chromosomes had long intercentric regions, and it is therefore not surprising that over 90 per cent. of the separations showed evidence of twisting in this region.

Most of the dicentric chromosomes had different free arm ratios, indicating separate origin. However, dicentrics occurred in adjacent nuclei with equal free arm ratios but with intercentric regions of different lengths, indicating common origin and persistence through a double bridge-breakage-fusion cycle. One dicentric had an anaphase separation indicating unequal strength of the two centromeres. Sears and Camara (1952) report a dicentric chromosome in wheat with unequal centromere strength and they considered the weakness in the centromere an intrinsic property and not due to its position.

Highly polyploid nuclei

Failure of anaphase separation, due to the presence of bridges, followed by restitution results in highly polyploid nuclei. Unbroken bridges occurred at telophase and interphase in '*Phyllis Cox*'. Where the next mitosis is synchronized in the connected nuclei, anaphase separation may again fail and restitution results in the formation of highly polyploid nuclei. If the mitotic divisions in the connected nuclei are not synchronized the two daughter nuclei each attempt anaphase separation and the result is four nuclei held together by connecting strands (Pl. II, Fig. 6).

Micronuclei

Micronuclei resulted from the non-inclusion of fragments in the telophase nuclei. In several endosperms of '*Phyllis Cox*' and one of *L. regale* numerous small globules, resembling micronuclei, and long irregular strands of Feulgen positive material occurred in areas adjacent to the point of entry of the vascular system into the ovule. The small globules were often attached to the main nucleus by fine connecting strands and they can retain their attachment to individual chromosomes during mitosis (Pl. II, Fig. 9). These globules resemble inactive micronuclei, but it is more likely that they are analogous to the globules of desoxyribose nucleic acid described by Cooper (1952) which pass from the tapetum to the developing pollen mother cells. The globules were often associated with areas in the endosperm where extensive chromosome abnormalities had occurred, and they may have accumulated as a result of the reduced mitotic activity of the nuclei. These globules made investigations on the activity of micronuclei difficult. However, all active micronuclei are synchronized with the main nucleus.

Spindle abnormalities

Tripolar spindles occurred in '*Phyllis Cox*' (Pl. II, Fig. 4). Similar spindle abnormalities have been described in *Iris* endosperm (Jungers, 1931), and while not necessarily associated with the chromosome breakage are indicative of the instability of endosperm tissue.

Extent of chromosome breakage

The extent of chromosome breakage and reunion, expressed as chromosome breakage per total anaphases (Table II) is an indication of the effect of the different genotype of the individual endosperms on the development of chromosome breakage.

6. Discussion

The cycle of chromosome breakage which results in the accumulation of large numbers of abnormalities is probably initiated from single chromatid bridges at anaphase. Such bridges could result from spontaneous chromosome breakage, failure of the end gene to reproduce, or from the introduction of broken chromosomes into the endosperm (McClintock, 1941). Since there is no chromosome breakage at meiosis in these *Lilium* species and hybrids, this last possibility is unlikely. However, the possibility of abnormalities occurring in pollen grain divisions cannot be overlooked. The subsequent mitotic abnormalities could arise as a result of the breakage and reunion of these chromatid bridges.

The accumulation of abnormalities reduces the mitotic activity of the endosperm and it is less well able to nourish the embryo. The embryo shows no mitotic abnormalities and only fails after the endosperm fails. The variable seed set of these hybrids under different environmental conditions (cf. Preston, 1939) illustrates the effect of environmental conditions operating on an unstable system.

Chromosome breakage is of wide importance in the failure of interspecific hybridization. Kihara and Nishiyama (1932) report giant nuclei, irregular mitosis, nuclei fusing and degenerating in endosperm of the hybrid *Avena strigosa* ($n = 7$) × *A. fatua* ($n = 21$). Boyes and Thompson (1937) report varying degrees of abnormalities in the endosperm of the hybrid *Triticum durum* ($n = 14$) × *T. vulgare* ($n = 21$), *T. dicoccum* ($n = 14$) × *T. vulgare*, and

T. monococcum ($n = 7$) × *T. spelta* ($n = 21$). Cooper and Brink (1944) report chromosome bridges, dumb-bell-shaped nuclei, and highly polyploid nuclei in the endosperm of the hybrid *Hordeum jubatum* ($n = 14$) × *Secale cereale* ($n = 7$). In all of these cases mitotic divisions in the embryo were normal and failure of the endosperm brought about failure of the seed to develop.

In the *H. jubatum–S. cereale* cross, Brink and Cooper (1944) consider that abnormalities in the antipodal cells preceded and induced the abnormalities in the endosperm. It is, however, more likely that the endosperm abnormalities result directly from the genic unbalance in the endosperm tissue. The success of embryo culture techniques in a wide range of plants illustrates the widespread failure of the endosperm to support an otherwise normal embryo. These cases of endosperm failure are probably associated with chromosome breakage and reunion.

7. SUMMARY

Chromosome breakage and reunion occurs in the endosperm of *L. regale* and of a lily hybrid.

The abnormalities probably commence with single anaphase bridges. In the hybrid they accumulate and further chromosome breakage produces multiple bridges, ring chromosomes, dicentric chromosomes, and fragments. Restitution produces highly polyploid nuclei in which mass chromosome breakage occurs.

These abnormalities culminate in the failure of the endosperm and lead to the death of the embryo. This sequence is probably of wide importance in the failure of interspecific hybridization.

8. ACKNOWLEDGEMENTS

This work was done while at the John Innes Horticultural Institution, Bayfordbury, Herts., on a Studentship from the Commonwealth Scientific and Industrial Research Organization, Australia. I wish to express my thanks to Dr. C. D. Darlington, for facilities, helpful advice, and criticism and to Dr. A. Rutishauser for help on technique.

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DESCRIPTION OF PLATE II

Illustrating R. D. Brock's paper on 'Spontaneous Chromosome Breakage in *Lilium* Endosperm'.

Endosperms of Lily hybrid, '*Phyllis Cox*'. Fixation in acetic alcohol, staining with Feulgen.

Fig. 1. Early telophase showing single chromatid bridge and fragment. (× 930.)

Fig. 2. Anaphase showing criss-cross separation of a dicentric chromosome. (× 930).

Fig. 3. Anaphase showing chromatid bridge and interlocking of a dicentric chromosome. (× 930.)

Fig. 4. Metaphase showing tripolar spindle. (× 930.)

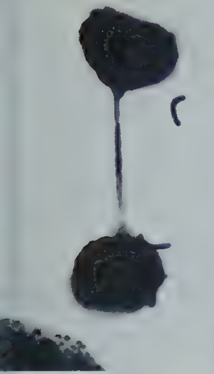
Fig. 5. Early anaphase of a highly polyploid nucleus showing acentric interlocked ring. (× 930.)

Fig. 6. Four nuclei connected by unbroken bridges. First mitotic division 'horizontal', bridges failed to break. Second mitotic divisions 'vertical' and not synchronized. (× 930.)

Fig. 7. Early anaphase showing mass spontaneous chromosome breakage. (× 670.)

Fig. 8. Late anaphase showing unbroken bridges, rings, and fragments. (× 930.)

Fig. 9. Metaphase showing globules of desoxyribose nucleic acid attached to the chromosomes. (× 930.)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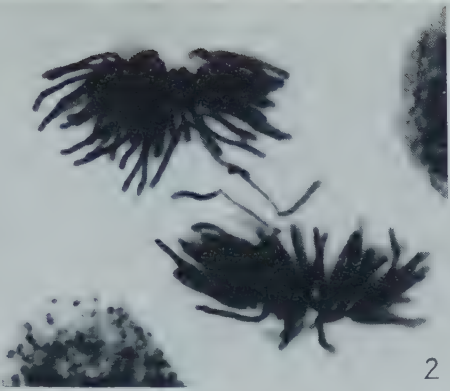
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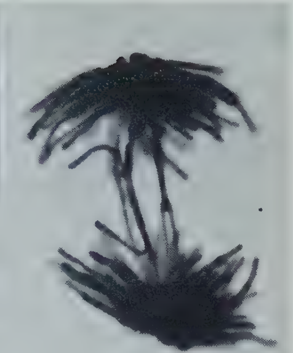
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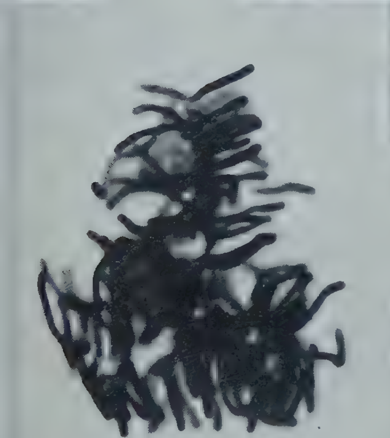
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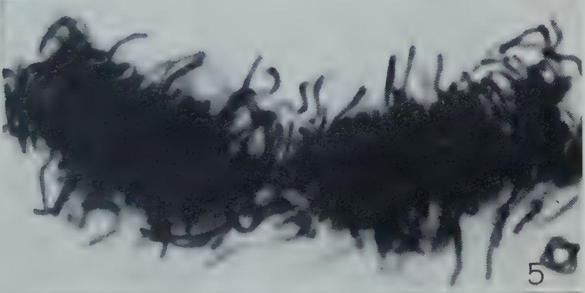
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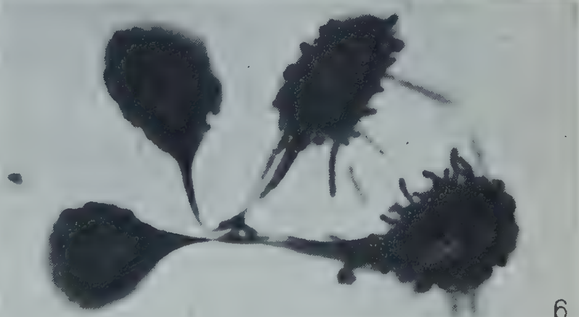
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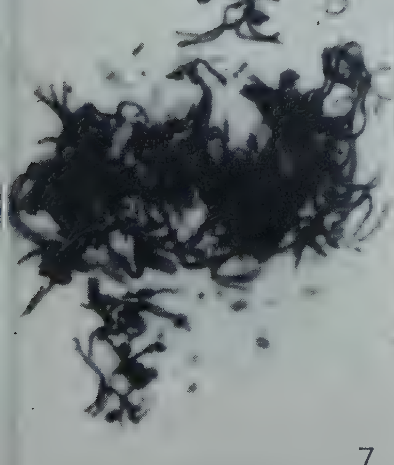
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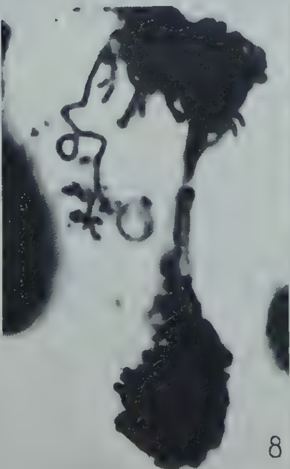
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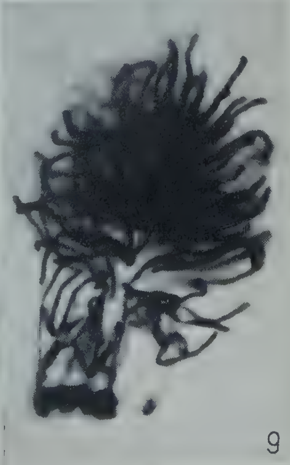
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R. D. BROCK

Physiological Studies in Plant Nutrition

XVII. A General Survey of the Free Amino-acids of Barley Leaves as affected by Mineral Nutrition, with Special Reference to Potassium Supply

BY

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With two Figures in the Text

Abstract

A survey by means of paper chromatography of the free amino-acids in barley leaves at different stages of development is described. The plants were grown in sand culture, using a variety of nutrient solutions, the most important variable being potassium supply. In general, the amino-acid distribution is not greatly affected by nutritional differences.

Potassium deficiency results in much increased contents of the amides, especially asparagine, and reduced amounts of the corresponding acids. Other acids increased under these conditions are serine, leucine, tyrosine, and phenylalanine, but the most characteristic changes occur in the basic constituents; lysine and arginine are increased, and an unknown basic substance accumulates very characteristically. Accumulation of this substance (probably an amine) is checked by the presence of either sodium or rubidium in the culture solution, almost as effectively as by increased potassium supply. Extreme potassium deficiency yields chromatograms closely resembling those from more moderately deficient plants.

When a low rubidium supply accompanies potassium shortage the disturbances occasioned by potassium deficiency, both in growth and in amino-acid distribution, are diminished, but a higher rubidium supply leads to toxic symptoms in the plant; large increases in the contents of the two amides and of their parent acids are then found, this being the only condition encountered that leads to simultaneous accumulation of both these types of substances.

Introduction

POTASSIUM deficiency is well known to result in the accumulation of free amino-acids in plants, but in the past owing to the magnitude of the task, fractionation of these amino-acids received very little attention. It seemed worth while therefore to apply the method of paper chromatography to the ninhydrin-reacting substances present in protein-free extracts of barley leaves, and so to extend the survey of effects of potassium deficiency in nitrogen metabolism begun here many years ago (cf. Richards and Templeman, 1936).

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The external symptoms of deficiency in this plant have been found to differ considerably with the general composition of the culture solution, e.g. the amount of calcium and sodium present, and the ionic form of the nitrogen supplied (Shih, 1936; Richards, 1941). Since nitrogen metabolism is clearly deranged by potassium deficiency, it is desirable to know whether the type of disturbance is constant or varies along with the external symptoms in accordance with the composition of the culture solution used. Further, it is known (Richards, 1941) that growth under potassium deficiency may be much improved by supplying rubidium, although this element itself induces characteristic toxic symptoms in the plant. In the investigation to be described, therefore, an endeavour was made to determine broad effects on the individual free amino-acid contents of various types of potassium-deficient barley, both in the presence and absence of rubidium. The rubidium was given at two levels in order to discriminate as far as possible between its beneficial and its toxic effects.

EXPERIMENTAL METHODS

Barley, var. 'Spratt Archer', was grown in sand culture in the open during the summer of 1950. Nine seeds were sown in each 10-in. pot on May 4, and nutrient salts applied in solution (as in all previous work from here[1]) on May 9. On May 11 the seeds germinated, and about 2 weeks later the plants were thinned to 3 per pot. In all, 123 pots were sown, comprising 14 nutritional treatments.

Three main types of solution were employed: (1) a sodium type in which the nitrate and phosphate were given as sodium salts and no excess calcium was present; (2) a calcium type containing no sodium, but with nitrate and phosphate supplied as calcium salts; and (3) an ammonium type, with nitrate and phosphate given as ammonium salts, and containing little calcium and no sodium. These three are standard solution types extensively used in previous experiments with barley in this institute. Further, three levels of potassium were investigated, designated K_1, K_3, and K_5, in which the relative amounts of this element were 1, $\frac{1}{9}$, and $\frac{1}{81}$, respectively; K_1 is a level designed to give optimal growth. To some of the potassium-deficient pots either sodium or rubidium was added in such amounts that the sum of the added element and the potassium present was equivalent to the potassium content of the next higher level (except where low rubidium was added to the K_3 treatments). Table I shows the various nutritional treatments, together with the number of pots in each. The symbols used there to denote the treatments are composed as follows. The solution type is indicated first and followed by a colon, after which are shown the potassium level and any addition of sodium or rubidium. Thus the symbol Ca:K_3Na stands for the calcium type of solution, potassium at the K_3 level, and sodium equivalent to the difference in potassium between K_1 and K_3; Rb and rb are used respectively to designate high and low rubidium application.

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[1] It is stated incorrectly by Hewitt (1952) that in the sand-culture work at this institute the nutrient salts were added in solid form.

TABLE I

Nutritional Combinations and the Number of Pots of each

Solution type.	Levels of alkali metals						
	K_1	K_3	K_3Na	K_3Rb	K_3rb	K_5	K_5rb
Na . . .	6	15	—	—	—	—	—
Ca . . .	6	15	8	1	5	—	—
NH_4 . .	12	15	8	1	5	20	6

Table II gives the amount of each salt added per pot, i.e. per 3 plants. To all pots were also added $FeCl_3$ as required and 0·01 g. $MnSO_4.4H_2O$.

From each treatment leaves were collected once or twice a week, always between 9 and 10 a.m. Samples were taken from treatments $NH_4:K_1$, $NH_4:K_3$, $NH_4:K_5$, and $NH_4:K_5$rb as soon as the first leaf was fully expanded (May 24), but in the other treatments sampling did not start until the middle of June. The last sampling occasion for all treatments was at the end of July. Each sample consisted of the last fully expanded leaf lamina from the larger shoots, enough material being collected to give 1·5 g. fresh weight. Dead parts of the leaves were excluded. The various samples from one treatment thus represented progressive stages in the development of the plants.

TABLE II

Nutritional Scheme

1. *Basal nutrients* (g. per pot)

Na		Ca		NH_4	
$NaNO_3$ . .	9·10	$Ca(NO_3)_2.4H_2O$.	12·64	NH_4NO_3 .	4·00
$Na_2HPO_4.12H_2O$.	0·13	$CaH_4(PO_4)_2.H_2O$	0·89	$(NH_4)_2HPO_4$.	0·05
NaH_2PO_4 . .	0·80	$CaCl_2.6H_2O$.	0·50	$NH_4H_2PO_4$.	0·77
$CaCl_2.6H_2O$.	0·50	$MgSO_4.7H_2O$.	1·25	$CaCl_2.6H_2O$.	0·50
$MgSO_4.7H_2O$.	1·25			$MgSO_4.7H_2O$	1·25

2. *Potassium levels* (g. per pot)

	K_1	K_3	K_5
K_2SO_4 . . .	1·85	0·21	0·023

3. *Other alkali metals* (g. per pot)

	Na	Rb	rb
$Na_2SO_4.10H_2O$.	3·04	—	—
Rb_2SO_4 . . .	—	2·52	0·28

Most of the results were derived from the plants described above, but some were obtained from plants grown in the preceding year. These plants were grown in the K_1 and K_3 solutions from each of the same three solution types.

As soon as possible the samples of leaves were ground in a mortar with sufficient alcohol to give a final concentration of about 75 per cent., allowance being made for the water in the leaf tissues (cf. Dent, Stepka, and Steward, 1947). Further alcohol (75 per cent.) was added as necessary during the grinding, which usually occupied 10–15 minutes. The material was then filtered on a small Buchner funnel and the residue washed with more 75 per cent. alcohol. The clear green extract (about 10 ml.) was evaporated to dryness under low pressure at 25–30° C., since tests showed that no detectable change in the amino-acid composition was induced by operating at this temperature as compared with evaporation in the cold. The dried extracts were stored in a desiccator until chromatographic analysis could be carried out.

The chromatographic procedure was essentially that of Consden, Gordon, and Martin (1944). Each extract was taken up in 0·15 ml. of water, giving a slightly viscous, dark yellow or brownish solution; 6 μl. was applied near one corner of a sheet of Whatman No. 1 filter-paper, giving a spot of 15 mm. maximum diameter. Two-dimensional chromatography was used, with water-saturated phenol as first solvent in the presence of HCN vapour, and as second solvent the 40–10–50 per cent. mixture of *n*-butanol, acetic acid, and water employed by Woiwod (1949). Tests using the two solvents in reverse order were unsuccessful. Several samples were re-chromatographed using collidine-lutidine-water in the second run, as recommended by Dent, Stepka, and Steward (1947). This solvent has the advantage of giving a better separation of the basic substances, which in the butanol solvent run into the areas occupied by the amides. The papers were developed using a spray of 0·1 per cent. ninhydrin in ethanol, and gentle heat.

Quantitative determinations were not attempted, but some indication of the relative amounts of the various amino-acids present was given by the sizes and colour intensities of the spots. The spots were graded into five intensity groups, from 'very faint' to 'very strong', these being 'scored' with the numerals 1 to 5. Sometimes it was necessary to insert intermediate groups, which accordingly were scored as 1·5, 2·5, &c. Results for the principal amino-acids are presented later in graphical form, but it should be understood that the scores are based upon subjective judgements and that any given score does not necessarily bear the same quantitative significance for the various amino-acids. From the method adopted the scores represent roughly a logarithmic series of values and for any one substance they give a good indication of its relative abundance under the different nutritional conditions, and also of its changing concentration with age of plant in any one treatment.

Most of the substances were identified by running pure amino-acids under the same conditions, e.g. by adding suspected acids to the samples. The map of their positions published by Dent (1948) was also very useful. The identity of the basic amino-acids was confirmed by the use of NH_3 in the cabinet during the phenol run, since under these conditions their R_F values are much increased. Extracts from a few samples were also hydrolysed by boiling in a reflux apparatus for 8 hours with 6N-HCl in order to determine whether peptides were present.

Inorganic ions, always present in the samples, caused considerable disturbance in many of the chromatograms, especially in the area occupied by glutamic acid, serine, and glycine, where a variable amount of streaking and overlapping occurred. They often increased the difficulty of assessing these substances, and it is possible that other substances present in small quantities may have been completely obscured. The technique of de-salting was not adopted.

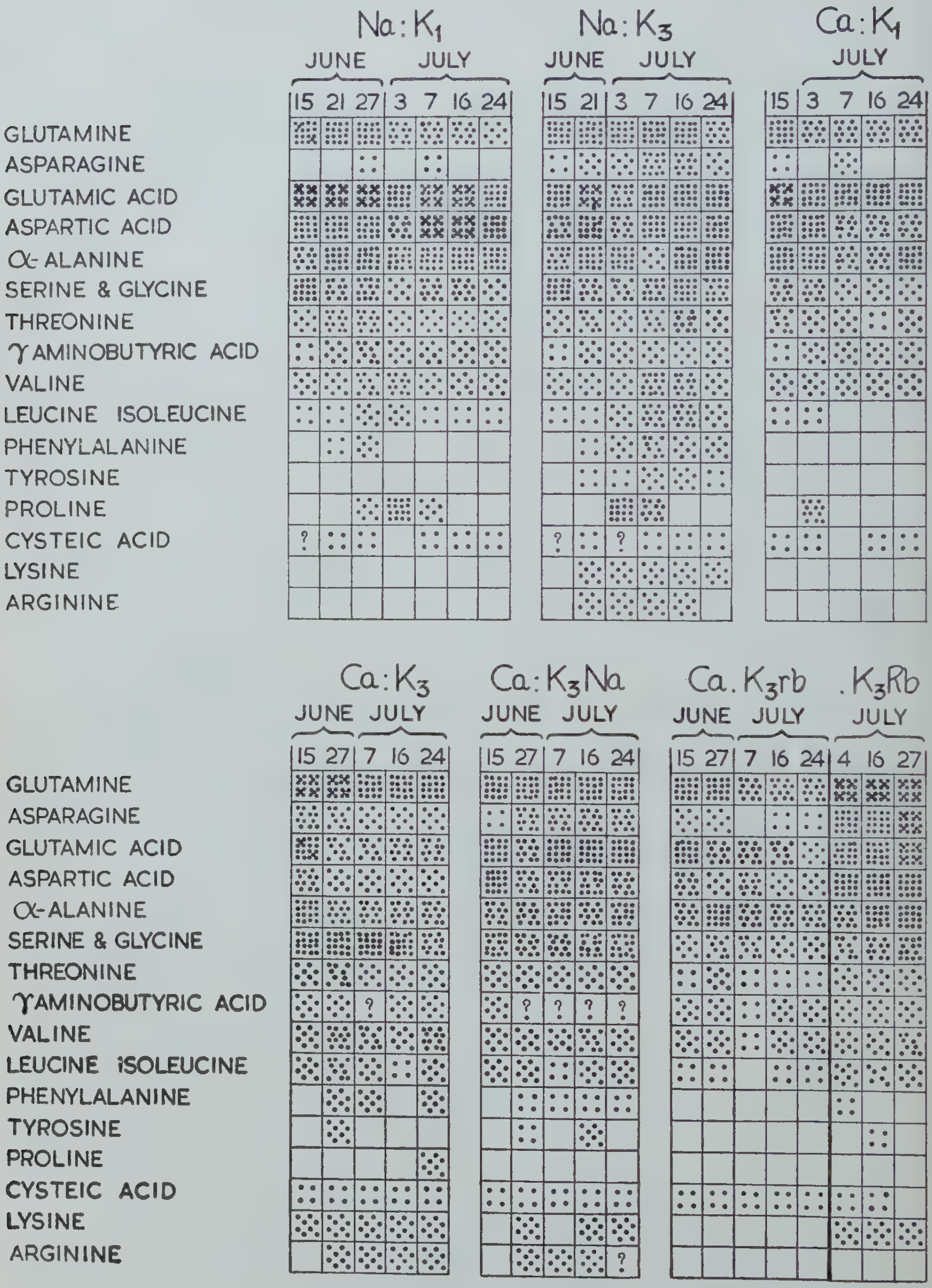
SYMPTOMS OCCURRING IN THE EXPERIMENT

The high-potassium plants (K_1) of all three solution types made vigorous growth and had a healthy appearance, although slight differences between them were noticeable. Plants of the ammonium series had at first a more spreading habit than those of the other two series, their tillers being less upright; shooting started somewhat earlier in $Ca:K_1$ and $NH_4:K_1$ than in $Na:K_1$.

Potassium-deficient plants differed markedly from the corresponding high potassium plants. In the $Na:K_3$ group the main distinguishing characters were light green leaves with high water contents, large numbers of tillers, and failure of ear emergence. Small brown areas were scattered over the leaves; this particular symptom is not, however, typical of potassium deficiency in high sodium conditions, for in most seasons similarly treated plants are conspicuously free from brown spots or specks. The $Ca:K_3$ plants were generally poorer in appearance, with fewer tillers and prematurely dying leaves. The lower leaves especially were heavily speckled with brown. These plants shot quite early, even before the $Na:K_1$ plants. In the ammonium type ($NH_4:K_3$) also the leaves died off very rapidly, although there was less brown speckling than on those of the calcium type.

The addition of sodium to these two last types improved their general appearance somewhat; the leaves were broader, especially in the calcium series, but in both $Ca:K_3Na$ and $NH_4:K_3Na$ shooting was delayed by the sodium addition.

The symptoms occurring in other treatments were very similar to those already described by Richards (1941). A discrepancy occurred, however, in the behaviour of the $NH_4:K_5$ plants. These were previously described as ceasing to grow in the beginning of June, when the leaves rapidly died off; many plants died completely during the next 4 weeks, but after considerable delay growth was actively resumed in the survivors. Later experience has shown that the severity of these toxic effects differs considerably with season, and in the present experiment they were comparatively mild, and indeed very few plants died. Also, the further growth of the survivors was resumed earlier than in the experiment described by Richards.


Na:K1
JUNE JULY
15 21 27 3 7 16 24
Na:K3
JUNE JULY
15 21 3 7 16 24
Ca:K1
JULY
15 3 7 16 24
GLUTAMINE
ASPARAGINE
GLUTAMIC ACID
ASPARTIC ACID
α-ALANINE
SERINE & GLYCINE
THREONINE
γ AMINOBUTYRIC ACID
VALINE
LEUCINE ISOLEUCINE
PHENYLALANINE
TYROSINE
PROLINE
CYSTEIC ACID
LYSINE
ARGININE
Ca:K3
JUNE JULY
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15 27 7 16 24
Ca.K3rb
JUNE JULY
15 27 7 16 24
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JULY
4 16 27
GLUTAMINE
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THREONINE
γAMINOBUTYRIC ACID
VALINE
LEUCINE ISOLEUCINE
PHENYLALANINE
TYROSINE
PROLINE
CYSTEIC ACID
LYSINE
ARGININE

Fig. 1

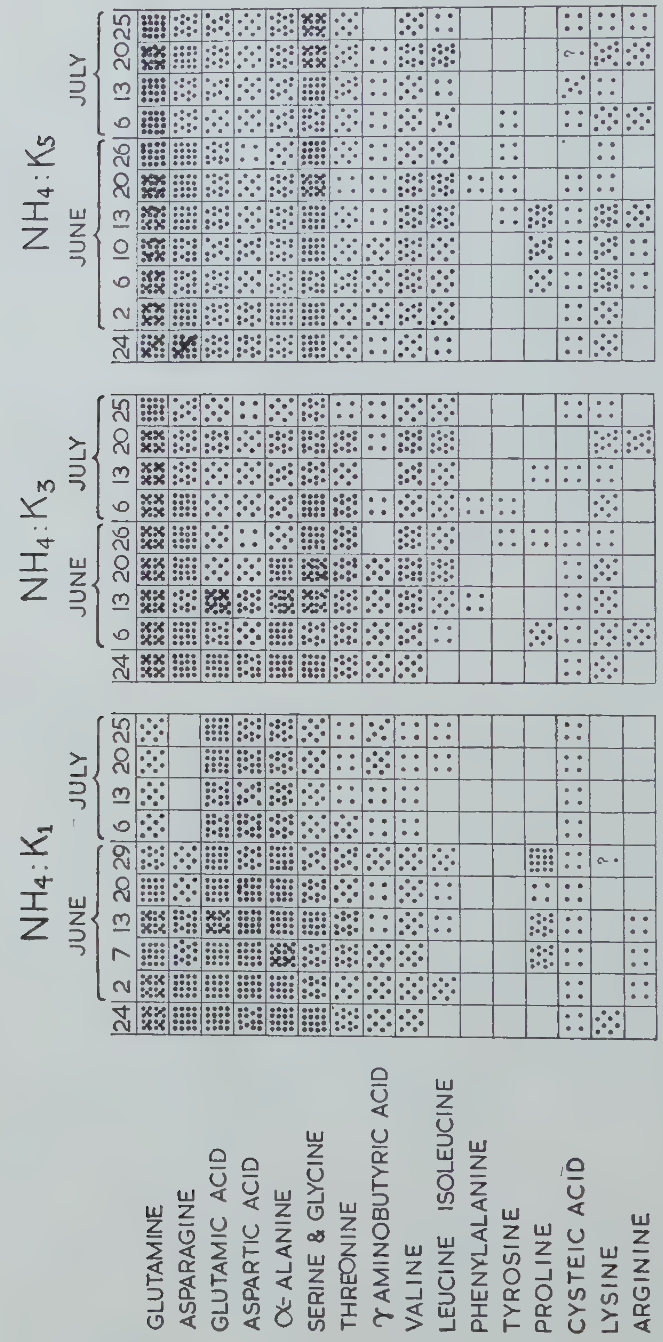
EXPERIMENTAL RESULTS

As has been mentioned, the leaf samples to be described were gathered between 9 and 10 a.m. To determine if any appreciable diurnal variations occur in the amounts of the several amino-acids, a single experiment was carried out wherein samples from plants grown with full nutrients (intermediate between Ca: K_1 and NH_4: K_1) were collected every 4 hours during a complete day. The only difference observed concerned proline; a small amount of this imino-acid was definitely present in the samples taken at 10 p.m., 2 a.m., and 6 a.m., whereas it could not be detected in samples gathered during the daylight period.

Method of graphical presentation. The relative amounts of the various amino-acids per unit fresh weight of leaf based upon scores are shown in Figs. 1 and 2. The results for each nutritional type are shown in a single block, and the headings of the blocks indicate the type of nutrient solution (Na, Ca or NH_4), the level of potassium supply (K_1, K_3, or K_5), and whether or not high or low Rb, or extra Na, had been given to the plants. The columns within blocks show the results of the analyses at different stages in the life-cycle and are headed with the date of sampling. The scores are indicated by the number of spots (4–20) entered: the blank spaces indicate that no ninhydrin spot could be detected. Where doubts as to presence or absence arose a query mark has been entered.

High potassium plants. At the earliest sampling-time common to the three high-potassium treatments, June 13–15, the contents in Na: K_1 and Ca: K_1 were very similar, but that in NH_4: K_1 was probably somewhat greater. The sodium and calcium series showed little change up to July 3, though a single paper from the former series on June 27 had indicated an increased content. After July 3, however, amino-acid content declined appreciably in the calcium plants, but much less, or not at all, in those from the sodium series. In the ammonium series a greater reduction occurred, the content here during July being the least of all. These results are generally similar to those obtained in the preliminary examination of the previous season, where also very early samples, consisting of material from the first and second leaves only, indicated that at the beginning of life-history a considerably greater content is found in plants given ammonium salts than in those supplied exclusively with nitrate. This presumably reflects the fact that ammonia enters the roots more rapidly than nitrate. As growth proceeds, therefore, in plants given ammonium nitrate the nitrogen round the roots becomes proportionally richer in nitrate; it must also become lower in total concentration than in plants supplied exclusively with nitrate. Hence the amount of nitrogen entering late in life may be reduced in the ammonium series, and as also vegetative growth in this series becomes particularly vigorous, these two facts may account for the reduced amino-acid contents here in the last leaves to be produced.

The relative contents of the amides are also interesting from the point of view of the two forms of nitrogen and their rates of uptake. Glutamine is


NH4:K1
NH4:K3
NH4:K5
JUNE
JULY
24
2
7
13
20
29
6
13
20
25
24
6
13
20
26
6
13
20
25
24
2
6
10
13
20
26
6
13
20
25
?
GLUTAMINE
ASPARAGINE
GLUTAMIC ACID
ASPARTIC ACID
α-ALANINE
SERINE & GLYCINE
THREONINE
γ AMINOBUTYRIC ACID
VALINE
LEUCINE ISOLEUCINE
PHENYLALANINE
TYROSINE
PROLINE
CYSTEIC ACID
LYSINE
ARGININE

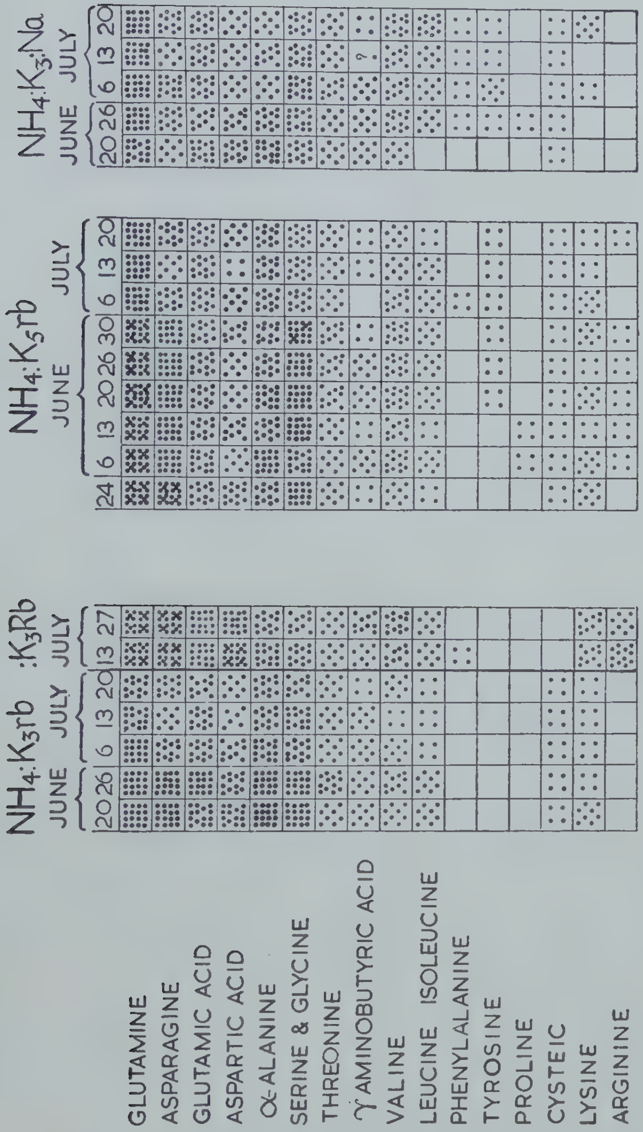

NH₄:K₃rb
:K₃Rb
JUNE
JULY
20 26 6 13 20
JULY
13 27
NH₄:K₅rb
24
JUNE
6 13 20 26 30
JULY
6 13 20
NH₄:K₃:Na
JUNE
20 26
JULY
6 13 20
?
GLUTAMINE
ASPARAGINE
GLUTAMIC ACID
ASPARTIC ACID
α-ALANINE
SERINE & GLYCINE
THREONINE
γ AMINOBUTYRIC ACID
VALINE
LEUCINE ISOLEUCINE
PHENYLALANINE
TYROSINE
PROLINE
CYSTEIC
LYSINE
ARGININE

FIG. 2

the characteristic amide in barley, and early in life-history gives one of the most intense spots on the chromatograms. Just as with total amino-acid, the glutamine content was highest in $NH_4:K_1$ in the early leaves, and the fall with age was greater than in the other series, so that finally these plants had the lowest contents of any. Asparagine was not usually detectable at all on papers from high potassium plants of the calcium and sodium series, though sometimes a very faint spot could be definitely identified. All early samples from the ammonium type of plant, on the contrary, contained high concentrations of this amide; during the second half of June the content declined rapidly, and in the last leaf or so none could be detected.

The production of asparagine in ammonium nutrition has been reported on several occasions, but its disappearance from the leaves of the present plants by early July is remarkable; this result nevertheless has been obtained in two seasons, and cannot be doubted. In well-nourished barley, asparagine appears to be produced in more than trace quantities only when free ammonia is entering the plant more rapidly than it can pass into the usual channels of metabolism. Deamination therefore appears normally to result in glutamine formation only, or almost exclusively. If asparagine is produced at all it must be broken down again immediately. The present results fully confirm the different status of the two amides in relation to the nitrogen cycle postulated by many workers.

The free dicarboxylic acids corresponding to these amides were both conspicuous on the papers from the high potassium samples. They apparently occurred in unusually constant amounts, not only as between the nutritional series but also among the successive leaves within any one series. In fact, in the sodium series there was no evidence of any fall in content in the later leaves relative to the earlier ones.

Of the mono-carboxylic acids, α-alanine was consistently present in highest concentration, and apart from the ammonium series there was little evidence of any decrease in later samples. Its hydroxy-derivative, serine, could not be adequately separated from glycine, so individual assessment was not attempted; there is evidence, however, that both these acids were present in all or nearly all the samples. As the plants aged, their combined contents declined considerably. The use of collidine-lutidine as second solvent in the previous year gave better differentiation in some ways between these two acids, and serine was most evident in papers from the calcium series of plants; glycine was often conspicuous in early leaf-samples, especially in the ammonium series, but later it almost or quite disappeared. Hydrolysis of the extracts resulted in considerably increased amounts of both substances.

A faint spot was given quite consistently by γ-amino-butyric acid, which appeared to be little affected by age. Threonine and valine were equally uniformly present, but showed more evidence of declining in amount in the later samples, especially again in the ammonium series. Leucine (and/or isoleucine) occurred in smaller concentrations, and on some papers, especially from the calcium series, only traces could be detected. Phenylalanine was found on two chromatograms from the sodium series, but usually no trace of it could be seen. Cysteic acid was consistently present, but always as a very faint spot; it is possible that some of the cystine from which it is derived was left behind in the leaf residue by the extraction method employed. Methionine sulphoxide was not identified on the main series of high potassium papers, but was sometimes present on those from all series of the previous season.

The behaviour of proline was different from that of all the other acids, since it appeared only on papers from the middle-leaf samples, but then might occur in quite high concentrations; this sequence was repeated in all three nutritional series. A very similar result was obtained in the previous season. It was mentioned above that in a series of observations designed to determine whether important diurnal changes occur, the only notable variations concerned proline, which was found in all the samples collected during the night period, but not in any gathered during daylight. Differences have also been found in the accumulation of proline in excised leaf laminae as between samples maintained alive for a few days in darkness, and others exposed to the natural alternations of daylight and darkness, but the quantitative differences have varied with occasion. In view of these sporadic observations, all of which indicate a highly individual behaviour, it would seem desirable to determine more precisely the conditions that lead to both the accumulation and disappearance of this substance.

Of the basic amino-acids, lysine and arginine were faintly visible on some of the early papers from the ammonium series; they were not detectable in the other nutritional series, which were always sampled later, though in the previous season they were occasionally found there also, again only in traces.

An unidentified substance occurred very usually on papers from the ammonium and sodium series, and in the preceding year at least this same substance was similarly present on papers from the calcium series. It appeared as a curiously small spot situated below aspartic acid, whether butanol-acetic acid or collidine-lutidine was used as the solvent in the vertical direction. Occasionally two spots were found, but it is probable that they represent the same substance, and that salt interference was responsible for the doubling. In spite of its small size, the spot may sometimes show quite a strong colour with ninhydrin.

Dent (1948) states that the tripeptide serylglycylglycine occupies the same position as glutamine on the chromatograms. The possibility of its occurrence in these extracts from barley leaves has been examined by extracting and hydrolysing the substances found in that position, and re-running the hydrolysate. In such trials glutamic acid was always recovered in large amounts, with lesser quantities of glycine and serine, and traces of some other undetermined substances. In one instance aspartic acid also was apparently recovered, but this may have been accidental. It therefore seems that Dent's substance is present, and this fact may make the assessment of glutamine from the spot intensity on the original chromatogram somewhat uncertain.

Potassium deficiency at the K_3 *level.* Reduction of potassium supply to one-ninth the standard amount greatly increased the total amino-acid content of the leaves, though the individual increases were not uniform. The general rise was greatest in the calcium series, but the absolute content under deficiency was no higher here than in the sodium series.[1] Well-defined maximum contents were attained at different times in the three series: in $NH_4:K_3$ in the middle of June, in $Ca:K_3$ apparently at the end of June, and in $Na:K_3$ not until the beginning of July. Late samples from the ammonium series had lower contents than those from the other two series, just as at the high potassium level.

Both amides were present throughout all samples in very large concentration. Glutamine was much more conspicuous than in the high potassium plants, being especially abundant in the ammonium series; little if any evidence of decline was found here in the late samples, at which time the contents of most other ninhydrin-reacting substances were diminished. Asparagine too was abundant everywhere, even in the sodium and calcium series where it is almost non-existent under conditions of high potassium supply, but again the highest concentrations occurred in the ammonium series. The content tended to decline in the late samples.

The corresponding dicarboxylic acids, on the contrary, both occurred in lower amounts than in the high-potassium plants. The decreases, though consistent, were comparatively slight in the sodium series, but quite substantial in the other two; as a result the absolute contents were considerably greater in $Na:K_3$ than in either $Ca:K_3$ or $NH_4:K_3$, especially during July. How far these decreases under potassium deficiency are accounted for by the increased amounts of the corresponding amides is a matter for speculation, but the correlation found everywhere may be suggestive of a simple relationship. Nevertheless it is possible to provide growth conditions under which these acids and their amides accumulate together relative to other amino-acids, as will appear when the plants grown with super-optimal amount of rubidium are described. The appearance of large quantities of asparagine in the sodium and calcium series under potassium deficiency is also interesting, in view of its virtual absence when potassium is plentiful. If it is produced only where ammonia tends to accumulate, its occurrence here might be ascribed to deamination proceeding more rapidly than the resulting ammonia can pass into the normal channel of protein synthesis. Under these same nutrient conditions protein is rapidly broken down in the mature leaves, where also nitrate tends to accumulate (cf. Richards and Templeman, 1936).

As a further result of the breakdown, some other amino-acids appeared in greater concentrations than in high potassium plants, notably glycine, serine, and leucine, and to a lesser extent valine also. These accumulations were general in all three solution types. Phenylalanine was usually present in Na:K_3 and Ca:K_3, and was seen in two papers from NH_4:K_3; in the high potassium plants it appeared on only two occasions, in the sodium series. Some amino-acids not normally detectable on papers from high potassium plants also occurred. Tyrosine was a usual constituent in series Na:K_3, and was seen occasionally in samples from the other two types. Owing to its slight solubility in 75 per cent. alcohol it may have been more abundant than appeared; its presence in high concentration under potassium deficiency has been reported (Mulder, 1949). Traces of β-alanine were seen in one sample from Na:K_3 and in three from the ammonium series, and on one or two papers of the preceding year (ammonium series) histidine also was found.

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[1] It should be stressed that the results described were obtained from samples of equal fresh weight; in terms of dry weight a rather different picture would be presented. In all three series investigated succulence is increased by potassium deficiency, so that on a dry-weight basis the general difference in amino-acid content between K_1 and K_3 would be exaggerated. The increase in succulence with deficiency is least in the calcium series and greatest in the sodium series.

Certain other amino-acids did not appear to increase in quantity with potassium deficiency. These included threonine (except for a possible slight increase in NH_4:K_3), α-alanine, γ-aminobutyric acid, and cysteic acid, though again, owing to the extraction technique adopted, the recoverable amounts of the last-named are uncertain. Proline on the whole appeared more sporadically than in high-potassium plants, and generally in lower concentration.

But the most striking effect of potassium shortage was on the basic ninhydrin-reacting substances. Lysine and arginine, normally scarcely detectable, appeared regularly in quite appreciable amounts in K_3 samples, except for those of the ammonium series, in which arginine occurred only infrequently. Another substance (or quite probably two substances with closely similar R_F values) was found apparently in higher concentration than these, in samples from the Ca:K_3 and NH_4:K_3 treatments, but in little more than trace amounts in Na:K_3. This substance is not well defined on chromatograms run with butanol-acetic acid as second solvent, but appears as a distinct spot when collidine-lutidine is substituted. In the previous year, when only the latter solvent was used, it was found to be consistently present even in very early leaf samples from the NH_4:K_3 and Ca:K_3 treatments. It increases in amount as the leaves turn yellow with age, but is not present (or if so only in trace amounts) in ageing leaves from high potassium plants. Like arginine and lysine, its R_F value is low in collidine-lutidine, while in phenol (using atmospheric air) it travels less rapidly than these amino-acids, taking up a position approximately under asparagine. When ammonia is present in the atmosphere its rate of movement is much increased, so that it now travels farther than lysine, closely behind the solvent front. It is assumed therefore that the substance is basic, and its behaviour in these solvents is reminiscent of that of some amines (Bremner and Kenten, 1951). The substance (or both, if there are two) is stable to hydrolysis with N-HCl. An attempt is being made to isolate and identify it.[1]

A few other unidentified ninhydrin-reacting substances sometimes appeared on papers from the potassium-deficient plants. In particular, a substance was present in most leaf samples which has a similar R_F in phenol to that of

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[1] See footnote to p. 32.

γ-aminobutyric acid and valine, and in both of the second solvents a rate rather lower than that of arginine; the position of this spot did not appear to be affected by the presence of ammonia in the phenol run. A trace of the substance was found also on two papers from high-potassium plants. Purple streaking (sometimes a more distinct spot) occurred on some papers from potassium-deficient plants, especially in the calcium series, indicating a substance with approximately the same R_F as valine in butanol-acetic acid and a rather lower value than valine in phenol. A third substance occurred on many papers from the calcium and ammonium series; this has an R_F similar to that of γ-aminobutyric acid or valine in phenol, and to that of threonine in butanol-acetic acid. It is possibly methionine sulphoxide, but the identification is not definite as yet.

Extreme potassium deficiency. The effects of extreme potassium deficiency were investigated in the ammonium series, primarily with the object of determining whether the severe toxic symptoms that often occur here are associated with characteristic changes in the individual amino-acid contents. The experiment was disappointing in two ways: first, the season proved to be one in which the toxic symptoms were relatively slight, and second, the amino-acid survey resulted only in chromatograms resembling very closely those already described from plants grown under less severe deficiency conditions. The accumulation of free ammonia that is known to occur in the $NH_4:K_5$ treatment, and is primarily responsible for the toxic effects, does not appear therefore to affect appreciably the free amino-acid distribution of these plants.

In the preceding year plants grown at the K_5 level in the sodium solution were also examined, in order to discover whether the unknown basic substance, characteristic of potassium deficiency in the calcium and ammonium solutions, will also accumulate in plants grown in the sodium solution if deficiency is extreme. The result was negative, and moreover the chromatograms were again closely similar to those derived from $Na:K_3$ plants.

Addition of sodium to $Ca:K_3$ *and* $NH_4:K_3$ *plants.* The addition of a limited amount of sodium to the $Ca:K_3$ plants generally made little difference to the contents of the various amino-acids. The amounts of aspartic and glutamic acids were, however, definitely increased to approximately the levels found in $Ca:K_1$. It is very probable that this change does not reflect to any extent a replacement of potassium by sodium in the metabolism of these acids, but should more properly be regarded as representing an approach towards the conditions found in $Na:K_3$; for in the later samples, at least, from the sodium series the dicarboxylic acids are present in higher amounts than in the calcium series, at both potassium levels. These two acids were also increased by addition of sodium to the $NH_4:K_3$ plants. Here both amides were also definitely decreased by the addition, this possibly being an important cause for the increase in the free acids; nevertheless, as a result of supplying sodium in the calcium series the dicarboxylic acids increased without any very definite change in the amide contents.

The basic amino-acids may have been somewhat reduced by the sodium additions, and clear indication of the unidentified basic substance was obtained in only one paper from these plants; this is in agreement with the fact that the substance occurs only in small amounts in potassium-deficient plants from the sodium series. Slight effects on other amino-acids (especially perhaps tyrosine and phenylalanine) following sodium addition may possibly be real.

Addition of rubidium. Under conditions of potassium shortage barley may benefit much from being supplied with rubidium. This effect is perhaps most striking in extreme potassium deficiency in the ammonium solution, where the severe toxic effects that often set in during early growth may be avoided altogether by addition of rubidium. Pronounced beneficial effects are obtained also when potassium is at the K_3 level, being then equally as great in the calcium and sodium series as in the ammonium series. But rubidium itself also exerts toxic effects whose severity increases with age; the symptoms it induces are quite characteristic (Richards, 1941). At a given potassium level the rubidium concentration optimal for total growth is therefore lower than that for maximal early growth. In the present experiments the lower of the two rubidium levels used is close to the optimum for total growth, while the higher level leads eventually to severe toxic derangements.

The object of this particular investigation was to determine whether either the beneficial or the toxic effect of rubidium is likely to be closely related to nitrogen metabolism. Effects of the lower level were sought on five occasions between mid-June and late July (K_3 *v.* K_3rb), but the effects of toxicity at the higher level (K_3Rb) were looked for only in July after severe external symptoms had developed, since the amount of plant material available was small. In addition, chromatograms were run from leaf samples of NH_4:K_5rb on nine occasions from May 24 onwards, to determine whether rubidium leads to marked changes in amino-acid metabolism in the ammonium solution type of extreme potassium deficiency, especially during early stages of growth.

(*a*) *Low rubidium.* Apart from doubtful indications of some reduction in the basic constituents and proline, no appreciable differences were induced in the free amino-acid composition by addition of rubidium to the NH_4:K_5 treatment. Although toxic effects due to this treatment were much slighter than usual, it nevertheless appears extremely probable that they neither depend on nor induce important changes in nitrogen metabolism, apart from the accumulation of ammonia itself. It was mentioned above that the chromatograms from NH_4:K_5 were also indistinguishable from those derived from treatment NH_4:K_3.

Where the same rubidium level was given to K_3 treatments, however, certain differences were evident in the resulting papers. In the ammonium series the departures from the K_3 type were always in the direction of the high potassium samples (NH_4:K_1) run during the same period (June 20–July 20). Increases over the amounts found in K_3, all of which brought the values closer to those of K_1, were found in glutamic and aspartic acids, α-alanine and γ-aminobutyric acid. These changes were offset by decreases, again in the direction of K_1, in glutamine, valine, leucine, and possibly also in lysine (the only basic substance found), threonine, and asparagine. There seems little doubt therefore that at this level of potassium in the ammonium series the derangement in amino-acid metabolism due to potassium deficiency may be counteracted to a quite considerable extent by supplying small quantities of rubidium, but that at extreme potassium deficiency (NH_4: K_5) the same quantity of rubidium still leaves the plants sufficiently starved to show no improvement in amino-acid distribution. It will be recalled in this connexion that increasing the potassium supply itself nine times (NH_4: K_3 *v.* NH_4: K_5) resulted in no noticeable difference in the chromatograms, and also that in treatment K_5rb the total alkali metal supplied was the same as in K_3. In so far then as the effect of low rubidium supply on the amino-acid distribution of these plants is concerned, there is no evidence that it differs in any way from that of potassium itself.

Addition of the same quantity of rubidium to treatment Ca: K_3 resulted in no spots on the chromatograms consistently heavier than those on corresponding papers from K_3. Considerable decreases, however, were caused in several substances, notably the two amides, the serine-glycine complex, threonine, valine, leucine, and also the unidentified substances present in K_3. No evidence at all was found of the presence of phenylalanine, lysine, arginine, and the unidentified basic substance, all of which were conspicuous on the K_3 papers. These reductions again resulted in chromatograms generally very similar to those of Ca: K_1, but in this calcium series the apparent concentrations of the two dicarboxylic acids were much closer to those in K_3 than K_1. On the other hand, the contents of the two amides were more similar to those of the high potassium plants than were those of NH_4: K_3rb to NH_4: K_1.

This difference in behaviour towards rubidium supply of the dicarboxylic acids and their amides, as between the calcium and ammonium series of plants, may well be exaggerated in these results, for equal fresh weights were used in the samples, and it is known (Richards, 1944) that the rubidium addition to treatment NH_4: K_3 causes a considerable reduction in water content, much greater than when it is added to treatment Ca: K_3. Therefore in these experiments the dry weights of the samples taken from NH_4: K_3rb may have been appreciably greater than in those from NH_4: K_3; that is to say, on a dry-weight basis the increases in the dicarboxylic acids (as also those in α-alanine and γ-aminobutyric acid) caused by rubidium addition to NH_4: K_3 were presumably less than actually appeared on the chromatograms, while the decreases in the amides were probably greater. However this may be, in both the calcium and ammonium series the low rubidium application resulted in a notable diminution of the characteristics of potassium deficiency found in the K_3 chromatograms, and in no appreciable evidence of new disturbances in amino-acid distribution accompanying its own toxic effects.

(*b*) *High rubidium.* The high rubidium additions were again made to treatments NH_4: K_3 and Ca: K_3; only a few comparisons, late in the vegetative stage, are available, but the main effects at this time are quite definite. These again concern the dicarboxylic acids and their amides, all of which increased to extremely high levels for the stage of growth. At this time the free acids in both K_3 treatments were reduced much below the concentrations found in the K_1 treatments, but in K_3Rb they were higher than in K_1; at this time, too, treatments K_3 and K_3rb had essentially similar contents of aspartic and glutamic acids, so that very different effects result from the different rates of application of rubidium. The glutamine and asparagine spots also were very heavy in K_3Rb, indicating higher concentrations than in any other treatment during July. Differential effects of the two rates of rubidium supply were even more in evidence here, for of the three treatments K_3, K_3rb, and K_3Rb, minimum amide contents were found in K_3rb.

The remaining amino-acids occurred in very similar amounts in treatments K_3 and K_3Rb, though the rubidium may have increased somewhat α-alanine and γ-aminobutyric acid, and possibly reduced the serine-glycine complex. The basic amino-acids were present, and these substances, together with valine and leucine, again show considerable evidence of a minimum concentration at the low supply level of rubidium. The unidentified basic substance characteristic of potassium deficiency in the absence of sodium has not been detected on a single paper from plants supplied with rubidium either at the high or low level.

The high rubidium chromatograms from the ammonium and calcium series were closely similar everywhere, pointing to the dominance in the results of effects of rubidium toxicity. A similar dominance of toxic effects in the external symptoms developed by these treatments was stressed previously by Richards (1941); potassium deficiency in barley leads to distinct plant types in the various nutrient series used, but when effects of high rubidium are superimposed there is a notable convergence to a quite new type of growth. It was argued that this fact is evidence against the deleterious effects of the element being due to its repressing potassium uptake, and therefore increasing potassium deficiency, and further evidence on this point may perhaps be found in the present results. For while potassium deficiency, like super-optimal rubidium application, leads to increased amide production, unlike rubidium it leads also to reduced concentrations of the dicarboxylic acids; effects of excess rubidium and of potassium deficiency on these acids are in opposite directions. Again, the increase of severity of potassium deficiency represented in the comparison $NH_4:K_3$ *v.* $NH_4:K_5$ causes no new phenomena in amino-acid composition, indeed the chromatograms are almost indistinguishable; but the addition of super-optimal rubidium to $NH_4:K_3$ leads to characteristic and large increases in the dicarboxylic acids and their amides, increases which are therefore unlikely simply to reflect greater potassium deficiency, but rather rubidium toxicity. Finally, the absence of the unidentified basic substance from any plants given rubidium may also suggest a similar interpretation, but, of course, since its accumulation is also depressed where sodium is present, it is possible that sufficient alkali metal of any kind may lead to a similar result.

CONCLUSIONS

The most salient fact that emerges from the above survey is unquestionably the general similarity of the free amino-acid pattern in barley leaves under widely differing conditions. None of the treatments investigated has provided evidence of large characteristic accumulations of any of the known amino-acids, apart from the dicarboxylic acids and their amides, nor of the absence of any normally present. Where known acids appear on chromatograms derived from potassium deficient plants, but are not usually to be seen on those from high-potassium plants, they occur only in very small amounts, and their presence may very probably be explained largely by the general higher concentration of soluble nitrogenous substances in the deficient plants, due to an increased rate of protein breakdown. They do not tend to accumulate, and so do not provide evidence of failure in specific enzyme systems. Other work, not reported above, would seem to indicate that a generally similar result is obtained even with phosphorus-deficient plants.

The only exception found in the work described is an unidentified substance, probably strongly basic, that occurs in those potassium-deficient plants not supplied with any alternative alkali metal; this substance apparently accumulates, and in samples of ageing leaves may reach quite high concentrations. It does not appear to be any of the usual basic amino-acids, but behaves on the chromatograms very similarly to some amines, for example, putrescine. Accumulation of the substance is apparently repressed almost as effectively by sodium or rubidium supply as it is by potassium.[1]

Amino-acids occurring in appreciably increased quantities under potassium shortage are lysine, arginine, glycine, serine, leucine, tyrosine, and phenylalanine, but the following are not usually increased: α-alanine, threonine, and γ-aminobutyric acid. More noteworthy variations are found in the dicarboxylic acids and their amides. With reduced potassium the free acids diminish and the amides increase, a result that may reflect simply the rapid proteolysis occurring here, increased deamination involving increased amide production. Asparagine, normally absent from high-potassium plants, is then produced in abundance. The same amide also appears in quantity at high-potassium levels if ammonium salts are provided round the roots, but with the cultural methods used it disappears again from the leaves before the end of the vegetative cycle. Glutamine is always present in quantity, but again increases in circumstances that induce the appearance of asparagine.

Extreme potassium deficiency brings about no changes not already evident under less severe shortage.

The addition of small quantities of rubidium to plants suffering moderate potassium deficiency improves their growth and leads to changes in the relative amounts of many amino-acids and the amides. These modifications are always in the same direction as would be caused by a further supply of potassium itself, and there is no evidence that this effect of rubidium in amino-acid metabolism differs in any way from that of potassium. Higher rates of rubidium supply induce toxic symptoms in the plants, and these are accompanied by large accumulations of aspartic and glutamic acids and their amides, without affecting much the concentrations of the remaining amino-acids. That two distinct effects of rubidium are primarily being investigated at the two levels of supply is indicated by the fact that among the three treatments (potassium deficiency, potassium deficiency+low rubidium, and potassium deficiency+high rubidium) well-defined minimum concentrations of some amino-acids, and especially of the amides, occur at the low supply level of the element. The results obtained at the high supply level are not similar to those induced by more severe potassium deficiency, hence the deleterious effects are not due simply to rubidium restricting potassium uptake.

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[1] Since this paper was prepared for publication this substance has been identified as putrescine (F. J. Richards and R. G. Coleman: Nature, clxx. 460 (1952)).

In these experiments the imino-acid proline has shown anomalous behaviour, but not in relation to the nutrient conditions under investigation. Further work designed to define the conditions that lead to its appearance in appreciable quantity might well be profitable.

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The Distribution of Plant Populations

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With one Figure in the Text

ABSTRACT

In studying the distribution of plant species in sample quadrats it may be assumed that the individuals are aggregated into groups showing a Poisson distribution, while the number of individuals in each group follows a logarithmic distribution. The resulting compound distribution of individuals per quadrat is the negative binomial. Evidence is produced to show that the distributions calculated on this basis agree with those actually obtained in the cases examined.

A GREAT deal of information on the distribution of various species in different plant associations has been obtained in recent years. In the first critical analysis of plant counts Blackman (1935) attempted to fit the Poisson series to observed distributions. Following his lead, other workers used this type of distribution as a basis for their calculations, and all found that, in the majority of cases, there was a significant difference between the observed results and the numbers expected, calculated on the basis of the Poisson series.

Archibald (1948) pointed out that the assumption of a random distribution of plant numbers did not agree with the views held by plant ecologists, and she showed that the distribution of some plants could be better described on the hypothesis of aggregation; i.e. that when one individual of a species is present, others of the same species are more likely to occur. In cases when a Poisson series would not fit the data, a good fit was often obtained by using the contagious series introduced by Neyman (1939). The characteristic feature of these series is the possibility of several modes, but unless such a set of modes is conclusively demonstrated one may reasonably feel reluctant to use the series.

In a later paper Archibald (1950) made use of the double Poisson series suggested by Thomas (1949) in her analysis of plant counts. The double Poisson series is based on the assumption that groups of individuals are distributed at random following a Poisson distribution, and the number of individuals in each groups was 1 plus an observation from a Poisson series with a different mean. No evidence, however, was given to suggest that the form of distribution of numbers of individuals in groups was of this type, so that there is also some reluctance to use this series.

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Where the null class cannot be observed (as in this case where each group must consist of at least one individual) it has been found that many ecological populations follow the logarithmic distribution first given by Fisher et al. (1943). If then the numbers of individuals in each group follow a logarithmic distribution and the number of groups in each quadrat follow a Poisson distribution, it can be shown (Quenouille, 1949) that the resulting compound distribution of numbers of individuals per quadrat is the negative binomial.

In addition, Anscombe (1950) has pointed out that the negative binomial distribution is 'biologically significant' in that it can be obtained by assuming that the progenitors are released with a uniform distribution in time and birth- and death-rates per individual are constants.

It is surprising then, that although the negative binomial has been applied to animals (Anscombe, 1949), it has not before been applied in detail to plant distributions. The differences found by other workers between the actual distributions and the theoretical distributions calculated on the basis of the Poisson, the double Poisson, and the contagious series of Neyman indicate that some other distribution should be more useful. It was therefore decided to fit the negative binomial to observed distributions, to determine whether this form was generally applicable to plant populations.

The data used in this paper were obtained by Steiga (1930) in an extensive study of the botanical composition of what he termed high and low prairie. In each area 40 quadrats of 1 square meter were examined, and the number of individuals counted for several species. In the case of grasses and sedges the basal area occupied by the different species in each quadrat was also measured. The data he collected have already been used by Clapham (1936) in an attempt to fit the Poisson series to the number of individuals per quadrat. From the two areas a total of 44 distributions were examined; 24 of these were for non-grassy species and the remaining 20 were for grasses and sedges. Of the former only 3 showed no great divergence from the Poisson series, and only 1 of the latter. These distributions have here been re-examined with a view to fitting the negative binomial series.

This series is defined by two parameters, the mean, m, and the exponent k. The expected frequency of zeros is

$$p_0 = \left(1+\frac{m}{k}\right)^{-k},$$

and the probability of observing any positive count r is

$$p_r = p_0 \binom{k+r-1}{r} \left(\frac{m}{m+k}\right)^r.$$

The variance of the distribution is

$$m+\frac{m^2}{k}.$$

The estimation of k by the method of maximum likelihood or minimum χ^2 is very tedious, but Anscombe (1949, 1950) has given several methods whereby

k can be estimated. One of the methods uses the variance of the sample. If this is equated to the population variance, then we obtain

$$k = \frac{m^2}{s^2 - m},$$

where s^2 is the sample variance; this is Method I in Anscombe's notation. In Method II the actual number of zeros obtained is equated to the expected number of zeros. In other words, k is chosen, by successive approximation, to satisfy the equation,

$$n_0 = N\left(1 + \frac{m}{k}\right)^{-k},$$

where N is the total number of samples taken. The remaining methods given are not so generally useful as these two, and only these have been used in the following calculations.

Non-grassy species

The twenty-four distributions were examined, and the values of the exponents were found by one of the above methods. In his paper Anscombe gives curves showing the efficiency of the two methods for various values of k and m. Roughly speaking, Method I is used when k is greater than unity, and Method II when k is less than unity. The χ^2 test was applied to each of the distributions, and only *Antennaria campestris* on low prairie showed any large disagreement between the actual results and the expected numbers calculated on the basis of the negative binomial series. The disparity is caused by a number of quadrats with 16 to 19 plants in each. This rise would suggest that a distribution with more than one mode would be more suitable. The double Poisson series and Neyman's contagious series were therefore fitted to the distribution; but the theoretical expectations were in neither case close to the actual results observed. There is therefore no added advantage in these two distributions even in this case. In view of the general agreement of the actual distributions with the distributions calculated using the negative binomial series, and as the same species on high prairie showed no great divergence from the negative binomial, it does not seem very likely that this exception represents a true deviation from the negative binomial. It is more probably due to the difficulty of counting individuals of a species in which individual plants form colonies by vegetative means.

There is some theoretical evidence (Kendall, 1948) to show that the value of k, the exponent, depends on the intrinsic power of the species to reproduce itself, and is independent of the external conditions of the environment. The value of k in the two communities should therefore, on theoretical grounds, be equal for each species.

This point has not been tested with actual data before, and it was felt that it would be instructive to determine values of the exponents for the combined distributions of the several species in both communities. Of the 24 distributions there were 7 pairs of species in high and low prairie (excluding the pair for *Antennaria campestris*). A combined value of *k* was then determined for each of the 7 species by using the total number of zeros found in the two communities.

TABLE I

Values of the Exponent of the Negative Binomial Distribution for Various Non-grassy Species in High and Low Prairie

Species . .	A	Am	C	E	H	P	S
Low prairie .	1·18	0·32	0·88	1·78	0·77	0·16	0·44
High prairie .	2·30*	0·30	0·77	0·69	0·40	1·62*	0·24
Combined . .	1·43	0·30	0·81	1·00	0·47	0·52	0·31

A	*Amorpha canescens*	H	*Helianthus scaberrimus*
Am	*Aster multiflorus*	P	*Psoralea floribunda*
C	*Cathartolinium sulcatum*	S	*Solidago glaberrima*
E	*Erigiron ramosus*		

* The values of *k* in these two cases were determined by Method I; the remainder were by Method II (see text).

Table I gives the value of the exponent for each distribution, together with the combined value for the two communities. Method II was used in all cases except for *Amorpha canescens* and *Psoralea floribunda*, both on high prairie. In these cases it was found from the graphs given by Anscombe that Method I should be more efficient.

The large-sample variance of *k* for Method I is

$$V(k) = \frac{2k(k+1)}{NX^2}$$

and for Method II it is

$$V(k) = \frac{(1-X)^k-1-kX}{N[-\log_e(1-X)-X]^2},$$

where N is the number of quadrats examined (in this case $N = 40$) and

$$X = \frac{m}{m+k}.$$

Using these formulae, the variance of *k* for each species in the two different communities was found. By an application of the *t*-test, *Psoralea floribunda* showed a difference between the two values which was significant at the 5 per cent. probability level; the remainder showed no great disagreement.

Table II gives the actual and the expected numbers of quadrats in the various classes for the different species in the two communities. The expected numbers for *Psoralea floribunda* were calculated using the two different values of *k* for the two communities, but those for the remainder were calculated using the mean value of *k* found from the total number of zeros in both areas. The appropriate mean number of species was used in each case. Application of the χ^2 test for goodness of fit showed no large deviations from

TABLE II

The Actual (column (i)) and Expected (column (ii)) Distributions for Non-grassy Species in High and Low Prairie

	Number of individuals	Number of quadrats in the different groups													
		A		Am		C		E		H		P*		S	
		(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)
LOW PRAIRIE	0	6	5·0	20	20·4	15	15·4	12	14·7	7	11·2	33	33·0	12	15·4
	1	6	5·5	6	5·5	10	8·6	7	9·3	6	4·9	3	3·7	2	4·5
	2	3	5·1	2	3·2	3	5·4	10	5·9	2	3·4	4	1·5	4	2·8
	3	6	4·5	1	2·2	5	3·5	5	3·7	6	2·6	0	0·8	3	2·1
	4	6	3·8	2	1·6	1	2·3	4	2·4	1	2·1	0	0·4	2	1·6
	5	4	3·2	2	1·2	2	1·5	1	1·5	3	1·7	0	0·2	1	1·4
	6	1	2·6	1	1·0	2	1·0	1	0·9	0	1·5	0	0·2	3	1·2
	7	1	2·1	1	0·8	1	0·7	0	0·6	3	1·3	0	0·1	0	1·0
	8	2	1·7	2	0·6	0	0·5	0	0·4	2	1·1	0	0·1	3	0·9
	9	0	1·4	0	0·5	1	0·3	0	0·2	3	1·0	0	0·0	0	0·8
	10	2	1·1	1	0·4	0	0·2	0	0·2	0	0·9	0	0·0	1	0·7
	11	0	0·9	0	0·4	0	0·1	0	0·1	0	0·8	0	0·0	1	0·6
	12	0	0·7	0	0·3	0	0·1	0	0·1	1	0·7	0	0·0	2	0·6
	Over 12	3	2·4	2	1·9	0	0·4	0	0·0	6	6·8	0	0·0	6	6·4
HIGH PRAIRIE	0	0	2·1	24	24·0	18	17·9	15	12·6	20	18·9	6	8·2	21	18·8
	1	5	2·6	5	5·9	10	9·1	5	8·6	6	7·1	12	8·3	4	5·3
	2	3	2·8	4	3·1	6	5·2	7	5·9	5	4·2	7	6·8	4	3·2
	3	2	2·8	1	2·0	3	3·1	3	4·0	4	2·7	3	5·1	1	2·2
	4	1	2·7	1	1·3	1	1·8	4	2·8	0	1·9	4	3·7	4	1·7
	5	2	2·5	3	0·9	0	1·1	1	1·9	1	1·3	3	2·6	0	1·3
	6	4	2·4	0	0·7	1	0·7	2	1·3	0	1·0	1	1·8	1	1·1
	7	2	2·2	0	0·5	0	0·4	0	0·9	1	0·7	1	1·2	0	0·9
	8	0	2·0	0	0·4	0	0·2	2	0·6	1	0·5	2	0·8	1	0·7
	9	3	1·8	1	0·3	0	0·2	0	0·4	0	0·4	0	0·5	0	0·6
	10	0	1·7	1	0·2	0	0·1	1	0·3	0	0·3	0	0·3	1	0·5
	11	2	1·5	0	0·1	0	0·1	0	0·2	0	0·2	0	0·2	0	0·4
	12	3	1·4	0	0·1	0	0·0	0	0·1	0	0·2	1	0·2	1	0·4
	Over 12	13	11·5	0	0·5	1	0·1	0	0·4	2	0·6	0	0·3	2	2·9

*Individual values of the exponent, *k*, used for the two communities.

the expected numbers. These results tend to support the theory that the value of *k* does not depend on the community associations.

In her paper comparing Neyman's series with the Poisson series Archibald demonstrated that the distribution of several species in which there was a significant divergence from a random distribution could be fitted reasonably well by Neyman's contagious series. These species, however, also showed no great divergence from the negative binomial species, and, in addition, the distribution of *Salicornia stricta*, which did not follow the Neyman series, gave good agreement with the negative binomial series.

Grasses and sedges

In view of the general applicability of the negative binomial series shown by the above examples it was thought that, in spite of the difficulty of segregation into separate individuals, the distributions given by Steiga should be tested on this basis. Table III gives the values of the exponents found for six species on both high and low prairie, together with the mean values obtained for the composite distribution in the two areas. The distribution of *Eragrostis pectinacea* on low prairie gave a variance smaller than the mean, so that the value of k is indeterminate. Although the Poisson series fits the distribution quite well, the use of the mean value of the exponent, i.e. 0·50, gives a distribution which also is not significantly different from the actual distribution obtained.

Table III

Values of the Exponent of the Negative Binomial Distribution for Six Species of Grass in High and Low Prairie

Species	Af	As	El	Ep	K	St
Low prairie . . .	8·50*	0·76	0·17	?	0·60	0·90
High prairie . . .	1·74*	1·70*	0·07	0·31	2·88*	0·55
Combined . . .	2·88*	1·37*	0·11	0·50	0·70	0·64

Af	*Andropogon furcatus*	Ep	*Eragrostis pectinacea*
As	*Andropogon scoparius*	K	*Koeleria cristata*
El	*Elymus canadensis*	St	*Stipa spartea*

* The values for k in these cases were determined by Method I; the remainder were by Method II (see text).

The large-sample variances of the various exponents were calculated using the formulae given above. The t-test showed that the differences between the two exponents for *Koeleria cristata* and *Andropogon furcatus* were significant. The remaining four species showed no significant differences between the two exponents, and Table IV shows the actual and the expected number of plants in the various classes calculated using the mean value of k in these cases. The table also shows the actual and the expected numbers of plants for the other two species, but the appropriate values of k were used. The χ^2 test indicated no great differences between the actual and the expected numbers.

The four species which have a common exponent in the two communities are *Eragrostis pectinacea*, which grows as distinct individuals, *Elymus canadensis* and *Stipa spartea*, which are tufted varieties of grass so that the identification of individuals is comparatively simple, and *Andropogon scoparius*, for which the individuals, although densely packed, can without great difficulty be separated. *Andropogon furcatus* is similar to *A. scoparius* in that dense communities are found. *Koeleria cristata* is more or less tufted in its growing habit, so that again individuals should not be too difficult to distinguish. These last three species are then slightly more difficult to deal with than the first three species, but in spite of that the distributions are found to follow the negative binomial series.

Three other species, viz. *Carex meadii*, *Bouteloua curtipendula*, and *Poa pratensis*, were also enumerated for the two areas. All of these exhibit a creeping type of growth, so that individuals are very difficult to distinguish. It is not then surprising that they show large differences between the actual and the expected distributions calculated on the basis of a negative binomial series. Faced with this serious difficulty of being unable to distinguish

TABLE IV

The Actual (column (i)) and Expected (column (ii)) Distribution for Grasses in High and Low Prairie

	Number of individuals	Number of quadrats in the different groups								Number of individuals	No. quadrats As		No. of individuals	No. quadrats Af*	
		El		Ep		K*		St							
		(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)		(i)	(ii)		(i)	(ii)
LOW PRAIRIE	0	25	28·1	28	30·7	13	13·0	7	9·5	0, 1	4	1·9	0–9	0	0·1
	1	4	3·0	10	6·3	3	6·6	2	5·4	2, 3	5	2·5	10–19	1	
	2	2	1·6	2	2·0	4	4·5	2	4·0	4, 5	1	2·5	20–29	0	
	3	2	1·1	0	0·7	3	3·3	7	3·1	6, 7	0	2·5	30–39	1	0·3
	4	0	0·8	0	0·2	5	2·5	2	2·6	8, 9	3	2·5	40–49	1	0·8
	5	1	0·6	0	0·1	3	1·9	4	2·1	10, 11	1	2·4	50–59	1	1·6
	6	1	0·5	0		1	1·5	4	1·8	12, 13	4	2·2	60–69	1	2·5
	7	1	0·4	0		3	1·2	2	1·5	14, 15	1	2·1	70–79	2	3·4
	8	1	0·4	0		1	1·0	3	1·3	16, 17	1	1·9	80–89	2	4·0
	9	0	0·3	0		2	0·8	1	1·1	18, 19	4	1·8	90–99	4	4·2
	10	0	0·3	0		1	0·6	0	1·0	20, 21	2	1·6	100–9	7	4·2
	11	0	0·2	0		0	0·5	2	0·8	22, 23	0	1·5	110–19	5	3·9
	12	1	0·2	0		0	0·4	1	0·7	24, 25	1	1·4	120–9	2	3·4
	13	0	0·2	0		0	0·4	1	0·6	26, 27	0	1·3	130–9	5	2·9
	14	0	0·2	0		1	0·3	0	0·5	28, 29	1	1·2	Over 140	8	8·7
	15	0	0·1	0		0	0·2	0	0·5	30, 31	3	1·0			
	Over 15	2	2·0	0		0	1·3	2	3·5	Over 31	9	9·7			
HIGH PRAIRIE	0	33	30·3	27	25·0	2	0·5	7	5·7	0, 1	2	1·4	0–3	4	1·2
	1	3	3·1	7	7·6	2	1·0	4	3·5	2, 3	4	1·8	4–7	1	2·2
	2	1	1·6	3	3·5	0	1·6	2	2·7	4, 5	1	2·0	8–11	5	2·7
	3	0	1·0	0	1·8	3	2·0	1	2·3	6, 7	2	2·0	12–15	1	2·9
	4	0	0·7	2	0·9	2	2·4	3	2·0	8, 9	1	2·0	16–19	3	2·9
	5	0	0·5	0	0·5	1	2·6	5	1·7	10, 11	2	2·0	20–23	2	2·8
	6	0	0·4	0	0·3	1	2·7	1	1·6	12, 13	1	1·9	24–27	3	2·7
	7	0	0·4	0	0·2	3	2·7	0	1·4	14, 15	0	1·8	28–31	0	2·5
	8	1	0·3	0	0·1	2	2·6	1	1·3	16, 17	2	1·7	32–35	1	2·3
	9	0	0·2	0	0·1	4	2·5	0	1·2	18, 19	1	1·7	36–39	2	2·1
	10	0	0·2	1		2	2·3	1	1·1	20, 21	3	1·6	40–43	2	1·9
	11	0	0·2	0		1	2·1	1	1·0	22, 23	1	1·5	44–47	2	1·7
	12	0	0·1	0		1	1·9	0	0·9	24, 25	2	1·4	48–51	0	1·5
	13	0	0·1	0		4	1·7	0	0·8	26, 27	3	1·3	52–55	2	1·3
	14	1	0·1	0		1	1·6	0	0·8	28, 29	0	1·2	56–59	1	1·2
	15	0	0·1	0		2	1·4	0	0·7	30, 31	0	1·1	60–63	2	1·0
	Over 15	1	0·7	0		9	8·4	14	11·3	Over 31	15	13·6	Over 63	9	7·1

* Individual values of the exponent, k, used for the two communities.

individuals reasonably easily, some other form of estimating the plant density must be employed. Two other methods of measuring the relative abundance of grassy species of this type have been used by various workers: (i) the area of ground covered by each species, and (ii) the productivity of each species. Plant density, however, should refer to the 'stand', or thickness of plants upon the ground surface, and therefore the first method should be more comparable with the enumeration of individuals. In addition, the productivity of each species is a measure of the yield at the time of sampling only, and will depend upon the immediately preceding climatic conditions. This will then be of little use in comparing the changes in a pasture over a period of treatment. On the other hand, the basal area covered by the species will not fluctuate to the same extent with the climatic conditions, and it will therefore be a better criterion for determining changes in the pasture over a long period. West (1937), and more recently Murray (1946), have given descriptions of various methods of obtaining estimates of area, and Murray recommends the Point Quadrat method of determining percentage area described by Levy and Madden (1933). Steiga estimated the percentage area covered in each qaudrat by plotting the different species on squared paper. These figures have been used in the following analyses.

It is to be expected that the distribution of the fraction of area occupied will follow the Pearson Type I curve. This is defined by two parameters l and m, and the ordinate of the curve is given by the equation

$$y = Ax^{l}(1-x)^{m},$$

$$\text{where } A \text{ is a constant} = \frac{1}{B(l+1,\ m+1)} = \frac{\Gamma(l+m+2)}{\Gamma(l+1)\Gamma(m+1)}$$

and x is the fractional area covered by the species ($x = 0$ when none of the species is present, and $x = 1$ when the whole area is covered by that species). The mean of this distribution is

$$\bar{x} = \frac{l+1}{l+m+2},$$

and the variance is

$$\sigma^2 = \frac{(l+1)(m+1)}{(l+m+2)^2(l+m+3)}.$$

The mean value of x^2 is given by

$$\overline{x^2} = \frac{(l+1)(m+1)}{(l+m+2)(l+m+3)},$$

and this together with the formula for the mean was used to determine the values of l and m. Three species were tested in this manner: *Carex meadii*, a creeping sedge; *Poa pratensis*, a creeping grass; and *Andropogon furcatus*, which has already been shown to follow a negative binomial series in the distribution of numbers of plants. Table V gives the values of l and m calculated from the above expressions, and Fig. 1 gives diagrams of the theoretical curves using these values, together with histograms of the actual numbers obtained.

TABLE V

Values of the Constants l and m for the Pearson Type I Curve, governing the Distribution of the Fractional Area covered by Two Grasses and One Sedge

	Andropogon furcatus		*Poa pratensis*		*Carex meadii*	
	l	m	l	m	l	m
Low prairie . .	1·1	21·7	1·3	28·3	0·7	1663
High prairie . .	0·1	63·9	−0·8	9·6	−0·3	167

The expected numbers of quadrats in the various groups were calculated from the formulae for an integral given by K. Pearson in Part II of his 'Tables for Statisticians and Biometricians'. Weddle's approximation to the integral over a given range is

$$\frac{3h}{10}(y_0+5y_1+y_2+6y_3+y_4+5y_5+y_6),$$

where $y_0,\ldots, y_6$ are the ordinates equally spaced over the range, at a distance h apart. This formula was used in all cases except for the initial range in the two cases where l was negative. In this case the method of continued fractions, also given by Pearson, was used. Application of the χ^2 test showed that there was no evidence of any large discrepancies between the expected numbers calculated by these methods and the actual numbers observed.

The areas given by Steiga for *C. meadii* on low prairie appear to have been rounded off to the nearest decimal place. As the species covered only a very small area in each quadrat, the data no longer resembles a continuous curve. It was thought better in this case therefore to plot the expected number of quadrats in which the area covered by the species was 0, 0·1, 0·2,... square decimetres. It was assumed that when the area was between 0 and 0·05 a zero area would be recorded; between 0·05 and 0·15 then a 0·1 area would be recorded, &c. This will tend to overestimate the number of recorded zeros, and underestimate the number of plots recorded with an area of 0·1 square decimetre, but even so, the agreement is reasonably good and χ^2 is not significant at the 5 per cent. probability level.

SUMMARY

Many workers have obtained large discrepancies between the actual distributions of plant species in sample quadrats and the expected distributions based on various theoretical series. In earlier papers it was assumed that the numbers of individuals followed a random Poisson distribution, but it was later pointed out that there is a tendency for aggregation into groups. If the number of groups then follows a random Poisson distribution, and if the number of individuals in each group follows the logarithmic distribution, it can be shown that the resulting compound distribution of individuals per

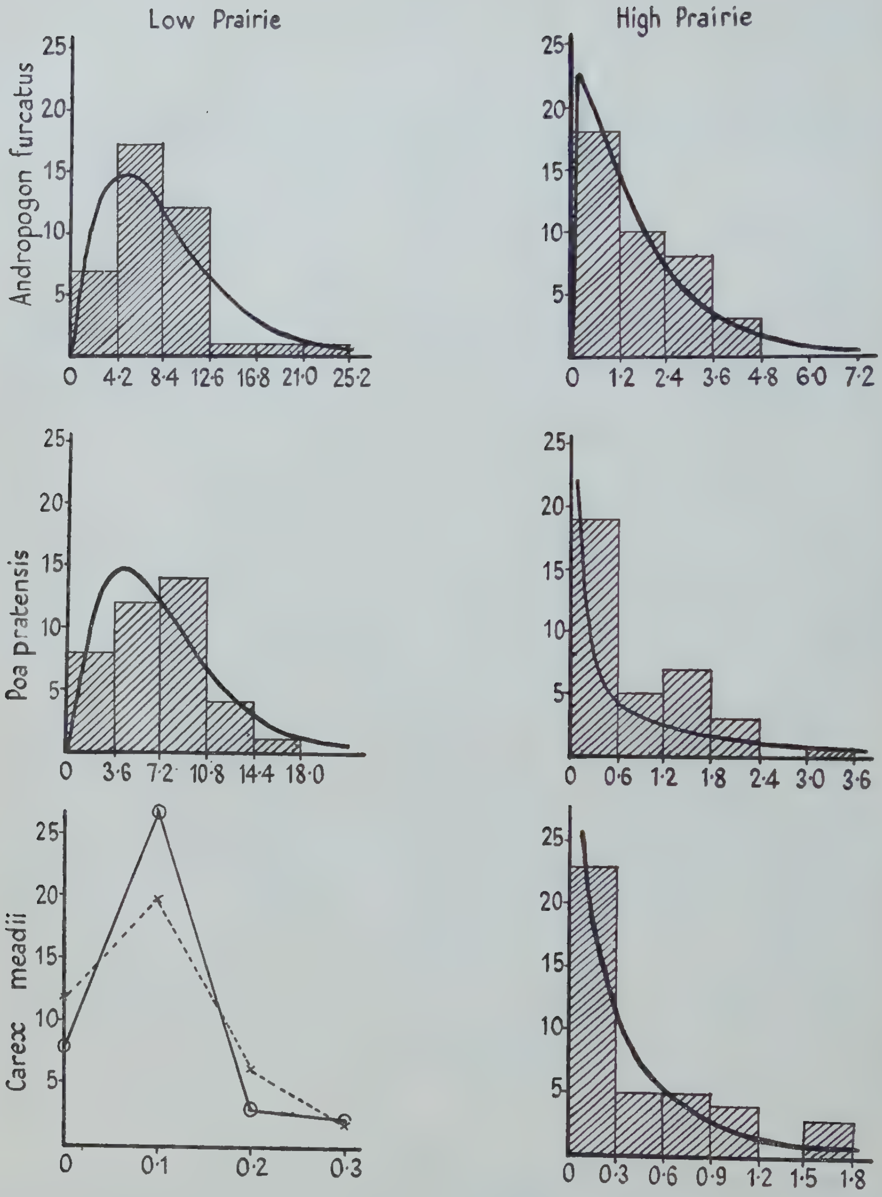

FIG. 1. Theoretical Curves (Pearson Type I), and Histograms of the actual distributions of the percentage area covered by two grasses and one sedge. (Percentage area on horizontal axis; number of quadrats on vertical axis.)

The figure for *Carex meadii* on Low Prairie shows the actual numbers of quadrats with 0, 0·1, 0·2, and 0·3 percentage cover (continuous line) and the expected numbers (broken line), assuming 0 to 0·05 be registered as zero; from 0·05 to 0·15 be registered as 0·1, &c.

quadrat is the negative binomial. Data collected by Steiga (1930) for two plant communities—high and low prairie—have been re-examined with a view to fitting this distribution. It has not before been used in an examination of plant communities, and it is shown that, in general, the expected distributions calculated on the basis of the negative binomial are in good agreement with the actual distributions obtained.

The value of the exponent of the negative binomial on theoretical considerations should be independent of the external conditions of the environment, so that for each species the exponent in the two communities should be the same. Empirical support for this hypothesis is obtained, for it is shown that in most cases a common value can be used to determine the distributions in the two communities. Where exceptions occur, the species is usually one for which it is difficult to separate individuals with any degree of certainty.

In the most difficult cases, as with a creeping type of grass, where it is impossible to distinguish individuals in the quadrat, it is suggested that some other form of measuring relative density be used; in particular, the fractional ground area covered by the different species is recommended. It is shown that the distribution of this measurement follows the Pearson Type I series.

The author wishes to thank Mr. B. Verdcourt for his helpful criticisms and for providing descriptions of the various species.

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Notes on Contagious Distributions in Plant Populations

BY

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ABSTRACT

Past experience has shown that the Poisson series is often inadequate as a model for describing plant populations. Various alternative two-parameter models have been suggested in place of the Poisson series, but they all depend on assumptions which may or may not hold. In this paper a different approach is put forward in that attention is concentrated on the mean number of plants per quadrat and an index of 'clumping' or 'contagiousness'. Examples are given as to the use of these concepts to test for differences between the distribution of a plant in two localities or between two plants in the same locality.

1. INTRODUCTION

SINCE Blackman (1935) attempted to fit the Poisson series to observed counts of plants in quadrat samples there has been a growing interest by botanists in the concept of randomness in a given community of plants of a particular species. This interest has been accompanied by an increasing number of statistical papers on the subject. The appearance of Mr. Robinson's interesting paper (1954) suggests that it may be valuable to both the botanist and the statistician to review the work that has been done and to attempt a synthesis of the objectives of the various investigations.

2. ASSUMPTIONS INHERENT IN A POISSON SERIES

It is well known that if in a long series of n independent trials the probability, p, of observing an event is constant and very small, then the probabilities of observing 0, 1, 2, . . . events are

$$e^{-\lambda}, \qquad \frac{\lambda e^{-\lambda}}{1!}, \qquad \frac{\lambda^2 e^{-\lambda}}{2!}, \ldots,$$

where $\lambda = np$.

This series, commonly called Poisson's series, was first produced by De Moivre in 1714 and the assumptions inherent in it may be stated, in the case of the quadrat sampling of plant data, as (i) the proportion of plants of a particular species in the community is small; (ii) the average proportion of plants of a particular species for any set of a large number of quadrats is approximately the same; and (iii) the probability of finding a second plant in a single quadrat after the first plant is observed is the same as the probability

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of observing the first plant. Although for most quadrat sampling (i) and (ii) are satisfied (iii) is not so easily satisfied.

3. ADEQUACY OF POISSON SERIES

The adequacy of the Poisson series may be tested for in one of two ways. In the first way an estimate of the Poisson parameter λ is made from the observed data and a test for goodness of fit made between the observed frequencies and the theoretical frequencies on the basis of this value of λ. A better test, however, consists of calculating the ratio

$$I = \frac{(\text{Observed standard deviation})^2\ (\text{Number of observations})}{\text{Observed mean}}.$$

As both the mean and the variance of the Poisson series are λ we should expect this quantity, I, to be about equal to the number of observations (Clapham, 1936). To judge significance we refer I, the index of dispersion, to χ^2 tables with degrees of freedom equal to one less than the number of observations. Too large a value of χ^2 will lead us to reject the Poisson hypothesis.

'Student' (1919) pointed out that the variance being greater than the mean is the usual cause of the Poisson hypothesis being inadequate for field data, and two mechanisms have since been put forward to 'explain' this inadequacy. The first mechanism assumes that the Poisson parameter, λ, varies over the field. In the second mechanism the Poisson parameter is assumed to remain constant but there is some form of dependence among the observations, a concept which Pólya (1930) called 'contagion' but we will refer to as 'clumping'. Feller (1943) has shown how it is impossible to distinguish between these two mechanisms on the basis of an observed set of data, and probably the true state of affairs lies in between the two and may well be different for each species.

4. COMPOUND POISSON SERIES

Various forms of two-parameter compound Poisson series have been suggested to take account of such departures from the basic assumptions as seem reasonable on the field experience. All these series have a variance greater than the mean. Anscombe (1950) lists such series, but we will discuss briefly three only.

i. *The negative binomial.* Greenwood and Yule (1920) appear to have been the first to show that if the Poisson parameter λ varies in a way that can be described by a Pearson Type III distribution (see, for example, M. G. Kendall, 1948, p. 142), then the resulting distribution is a negative binomial. No evidence has yet been produced regarding the propriety, or otherwise, of using the negative binomial, and the fact that it fits empirical data quite well at times, as Robinson remarks, is no justification of the Type III assumption. Since the work of Fisher (1941), Fisher et al. (1943), D. G. Kendall (1948), Anscombe (1949), and Quenouille (1949) all rests on this assumption it cannot be regarded as advocacy for the invariable fitting of the negative binomial to observed data.

ii. *Neyman's contagious distribution Type A.* This series was put forward by Neyman (1939) to describe the numbers of grubs in squares of a given area. It was based on the assumption that after a clutch of eggs was laid there would be clustering of the subsequently hatched grubs owing to their being able to move only a very short distance in a given time. Archibald (1948) applied this to botanical data by reasoning that we might imagine the seed travelling a short distance and then growing rather than the reverse. This, clearly, is an assumption that may or may not be justified by the physical evidence.

iii. *Thomas' double Poisson.* Thomas (1949) supposed that given one plant was found in a quadrat the probabilities of finding more were given by the terms of a second Poisson distribution. This assumption was made after consultation with Archibald, but it was not suggested that it was universally applicable.

5. Suitability of Compound Poisson Series

It is clear that a wide variety of two-parameter compound Poissons are possible and the appropriate series must be determined for each individual case. However, it is possible to say on occasions that certain series are unsuitable. Both Neyman's and Thomas' distributions have one mode usually but can have two and very occasionally more. It is not, however, possible for the negative binomial to have more than one mode. When an observed series has more than two modes it may be open to question whether some of the modes have been introduced by counting errors. For example, taking Robinson's *Solidago glaberrima* (low prairie) we have the following table:

Table I

Quadrat Counts of Solidago glaberrima

Number of individuals	0	1	2	3	4	5	6	7	8	9	10	Over 10	Total
Number of quadrats	12	2	4	3	2	1	3	0	3	0	1	9	40

The modes at 0 and 2 are undoubtedly real. They correspond to empirical series configurations observed by Archibald, Clapham, and others on diverse occasions with various species and there seems no reason why difficulties of counting enter into such quadrats. On the other hand, the mode at 6 may be questioned, as it might be argued that the three quadrats with six individuals arose through slight counting errors. The dip in the 1 group, however, is shown in no fewer than four of the fourteen series in Robinson's Table II, a fact which raises a query concerning his advocacy of the invariable use of the negative binomial.

6. Alternative Procedure

The difficulty of deciding the appropriate type of compound Poisson distribution to use raises the question as to whether it is necessary to fit any such distribution. Granted that the Poisson hypothesis has been rejected we usually want, as an alternative, to state the mean number of plants on the field and also some measure of the 'clumping' or 'contagiousness'. We would suggest that an appropriate index of clumping will be to calculate

$$I = \frac{\text{Observed variance}}{\text{Observed mean}} - 1.$$

These two quantities, the mean of the series and the index of clumping, will be entirely adequate to describe the data if a two-parameter compound Poisson of some type will be a satisfactory fit. The advantage of these two quantities is that it becomes possible to compare the degree of clumping in two species, or with the same species in two different localities. Let the series of the first species have N_1 observations with a mean λ_1 and a variance v_1 whilst the second series has N_2, λ_2, and v_2. We may first wish to know whether the mean number of individuals per quadrat is the same for both series. Assuming N_1 and N_2 large we would calculate

$$\frac{\lambda_1 - \lambda_2}{\left(\frac{v_1}{N_1} + \frac{v_2}{N_2}\right)^{\frac{1}{2}}}$$

and refer to normal curve tables to judge significance. But whether the means differ or not we may still wish to know whether the degree of clumping could be the same for the two species. If N_1 and N_2 are both equal to N and less than half the total frequency is in the first three groups, then we may calculate

$$w = -\tfrac{1}{2}\log_e \frac{v_1 \lambda_2}{\lambda_1 v_2} = -1{\cdot}15129 \log_{10} \frac{v_1 \lambda_2}{\lambda_1 v_2}.$$

The exact distribution of w under the assumption that the clumping in the two populations is the same is rather involved but large-scale computations have shown that if w lies outside the range

$$-2{\cdot}5/\sqrt{(N-1)} \quad \text{and} \quad +2{\cdot}5/\sqrt{(N-1)},$$

then it shows a significant difference between the indices of clumping for the two species. This procedure was suggested by Bateman's (1950) investigation of the distribution of the index of dispersion when the Poisson hypothesis does not hold, and we shall publish a fuller mathematical justification of the procedure elsewhere.

When half or more than half the total frequency is in the first three groups we would suggest replacing the above procedure by one based on a test recently put forward by Moore (1953). Instead of testing for the equality of the ratio of mean to variance in the two series, it is necessary to use a procedure which tests jointly for the equality of means and the equality of variances. Suppose that the proportionate frequency in the first three groups of the first species be r_0, r_1, and r_2 (out of a total sample of size N_1) and in the first three groups of the second species r'_0, r'_1, and r'_2 (out of a total sample of size N_2). We calculate

$$G = \log_e\left(\frac{r_0 r_2}{r_1^2}\,\frac{r_1'^2}{r'_0 r'_2}\right),$$

which is the logarithm of the ratio of the sample criteria of contagion. If the contagion is the same in the two populations from which the samples have been drawn, then we may assume G to be normally distributed with approximately

$$\text{Mean } (G)$$
$$= -\frac{1}{2}\left(\frac{1}{N_1}-\frac{1}{N_2}\right)(N_1+N_2)\left[\frac{1}{r_0 N_1+r'_0 N_2}+\frac{1}{r_2 N_1+r'_2 N_2}-\frac{2}{r_1 N_1+r'_1 N_2}\right]$$

and standard deviation

$$(\text{S.D. } (G))^2 = \left(\frac{(N_1+N_2)^2}{N_1 N_2}\right)\left[\frac{1}{r_0 N_1+r'_0 N_2}+\frac{1}{r_2 N_1+r'_2 N_2}+\frac{4}{r_1 N_1+r'_1 N_2}\right].$$

To carry out a test of significance we calculate therefore

$$\frac{G-\text{Mean } (G)}{\text{S.D. } (G)}$$

and refer to normal curve tables. It will be noticed that if $N_1 = N_2$ the mean of G is zero. For the test to be reasonably sensitive it is necessary for there to be at least 100 observations in each sample.

Another procedure which is really equivalent to that just outlined is the familiar χ^2 test, although this again will generally require a reasonable number of observations. Let there be frequencies n_0, n_1, n_2 in the first three groups of a sample of N_1 observations, and let n_3 be the number of observations in the fourth and subsequent groups so that

$$n_0+n_1+n_2+n_3 = N_1.$$

For a second sample of N_2 let the corresponding quantities be n'_0, n'_1, n'_2, and n'_3. The quantity χ^2 will be

$$\chi^2 = \frac{(n_0 N_2-n'_0 N_1)^2}{N_1 N_2(n_0+n'_0)}+\frac{(n_1 N_2-n'_1 N_1)^2}{N_1 N_2(n_1+n'_1)}+\frac{(n_2 N_2-n'_2 N_1)^2}{N_1 N_2(n_2+n'_2)}+\frac{(n_3 N_2-n'_3 N_1)^2}{N_1 N_2(n_3+n'_3)},$$

and it is distributed with 3 degrees of freedom. The 5 per cent. and 1 per cent. significance levels are 7·81 and 11·34 respectively.

7. ILLUSTRATION OF METHOD

As an illustration of the procedure we have outlined in the previous paragraph let us apply the tests to two examples. First we take the counts of *Erigiron ramosus* given by Robinson for both high and low prairie. It is

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doubtful whether there are enough observations for firm conclusions to be drawn, but we proceed for purposes of illustration:

$$\text{Low prairie } \lambda_1 = 1{\cdot}725 \qquad v_1 = 2{\cdot}499 \qquad N_1 = 40$$
$$\text{High prairie } \lambda_2 = 2{\cdot}175 \qquad v_2 = 6{\cdot}494 \qquad N_2 = 40$$

For the difference between the two means we have

$$\frac{2{\cdot}175-1{\cdot}725}{\left(\dfrac{2{\cdot}499+6{\cdot}494}{40}\right)^{\frac{1}{2}}} = 0{\cdot}95,$$

and we conclude, after referring to normal curve tables, that the observed difference in the two means could be due to chance. We now turn to possible differences in clumping, and since over a half of the frequencies lie in the first three groups we calculate

$$G = \log_e\left(\frac{12\times 10}{49}\,\frac{25}{25\times 7}\right) = -\,1{\cdot}0503.$$

$$\text{Mean } (G) = \text{zero},$$

$$\text{S.D. } (G) = 2\left[\frac{1}{37}+\frac{1}{17}+\frac{1}{3}\right]^{\frac{1}{2}} = 1{\cdot}295.$$

Thence
$$\frac{G-\text{Mean } (G)}{\text{S.D. } (G)} = -\,0{\cdot}81,$$

and we conclude that no difference exists in clumping between high and low prairie.

As a second example we take the distributions of *Salicornica stricta* and *Plantago maritima* given by Archibald (1948). For these we have

$$\textit{Salicornica stricta } \lambda_1 = 6{\cdot}99 \qquad v_1 = 27{\cdot}26 \qquad N_1 = 100$$
$$\textit{Plantago maritima } \lambda_2 = 5{\cdot}05 \qquad v_2 = 14{\cdot}39 \qquad N_2 = 100.$$

For the difference between the two means we have

$$\frac{6{\cdot}99-5{\cdot}05}{\left(\dfrac{27{\cdot}26+14{\cdot}39}{100}\right)^{\frac{1}{2}}} = 3{\cdot}01,$$

which is clearly significant. However, as less than half the observations fall in the first three groups, we may still use the test based on w to decide whether the clumping may be considered the same or different.

$$w = -1{\cdot}15129\log_{10}\left(\frac{27{\cdot}26\times 5{\cdot}05}{14{\cdot}39\times 6{\cdot}99}\right) = -0{\cdot}1569.$$

The value of N is 100, so that for w to be significant it would have to be less than $-2{\cdot}5/10 = -0{\cdot}25$, and hence our value is not significant. This latter form of test might be very useful in comparing the distribution of a species in different areas or at different seasons when the mean value may well change.

8. CONCLUSIONS

It is suggested that the tests we have described will, if applied consistently, enable the ecologist to describe his observed data without reference to compound Poisson distribution. This is an extremely fruitful procedure in that it eliminates the doubtful validity of results which are constantly based on unproved assumptions concerning the underlying form of the compound Poisson distribution. It would, however, be interesting to have enough data to be able to check these assumptions as to the form of the distributions because Fisher and others have shown that many important conclusions may be drawn provided the original assumptions are correct.

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Observations on the Symmetry of the Shoot in *Vicia faba* and some Allied Species, and on the Transmission of some Morphogenetic Impulses

BY

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With five Figures in the Text

ABSTRACT

Axillary buds occur in unsymmetrical arrangements so that any node must be either right- or left-handed. The two types occur in roughly equal numbers but are not randomly distributed. The condition of any node is determined partly by stimuli transmitted from the nodes below. The tissues of the stem can act as a filter, transmitting right- and left-handed stimuli with different degrees of efficiency. Although there are considerable interspecific differences in respect of these relationships, it is unlikely that the actual state of right- or left-handedness of a node is subject to any direct genetical control. In this respect the bud arrangements resemble phyllotactic spirals and differ from most other right- or left-handed structures occurring in plants.

INTRODUCTION

IN most alternate-leaved dicotyledons the first leaf of an axillary branch does not stand vertically above the axillant leaf but more or less to one side of it. It follows that in such a case any individual node presents an unsymmetrical configuration which is one member of a stereoisometric pair. Although this principle must be generally familiar, its implications, which are interesting and complex, seem never to have received serious consideration.

Vicia faba and some allied species offer a favourable field for the study of the phenomena involved. In these plants, as in most Leguminosae, each leaf-axil gives rise not to a single bud but to a whole series. The buds are placed in a descending (centrifugal) zigzag, each with its first leaf directed away from the median line of the group. The two possible arrangements are shown in Fig. 1, where the main stem is represented by a white lozenge and the axillant leaf by a white crescent. The number of buds in an axil is quite indefinite and can be artificially increased by various experimental treatments. For the present purpose it will be sufficient to show the first four in each instance; they are represented by black lozenges of graded size, the first leaf of each by a black crescent. The two nodal conditions will be denoted throughout by the letters R and L, according to the convention established in Fig. 1.

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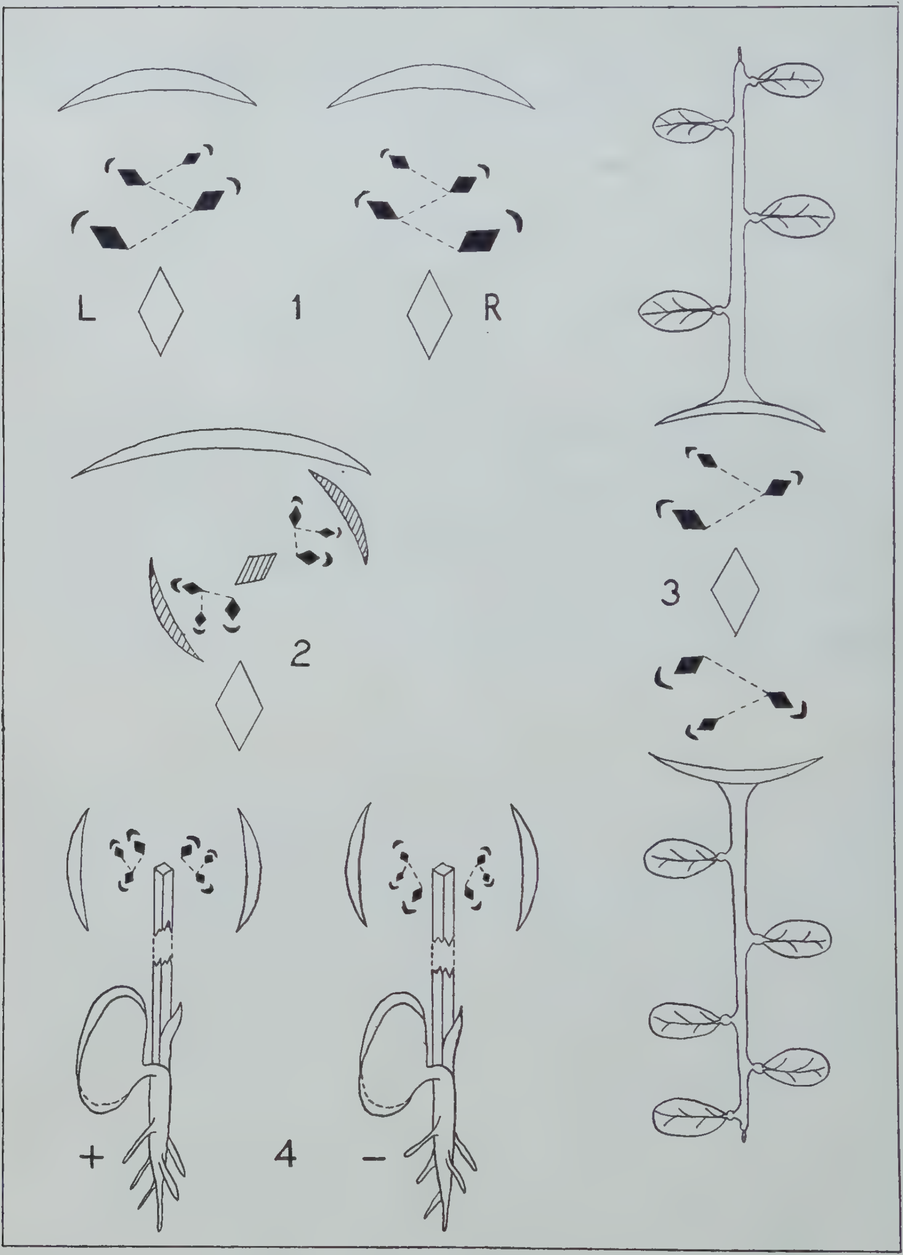

FIGS. 1–4. Fig. 1, diagrams of R and L nodes in plan; Fig. 2, nodes of an axillary shoot in plan; Fig. 3, two successive nodes showing relationship between arrangement of buds and arrangement of leaflets; Fig. 4, + and − seedlings, the lower parts shown in side view, the upper in plan.

ANALYSIS

The investigation now to be described was an attempt to discover what factors cause a node to assume one of the possible forms in preference to the other, and was concerned with *Vicia faba* (variety 'Claudia'), *Pisum sativum* (varieties 'Tall Sugar', 'Kelvedon Wonder', and 'English Wonder'), and *Cicer arietinum*. More than 500 plants of *Vicia* were examined, and about 100 plants of each of the other types. In all these plants the fundamental geometry of the shoot is the same, the leaves being arranged distichously throughout.

Nodes on axillary shoots. The simplest (and least interesting) condition is that in which the state of a node is entirely determined by what may be called topographical causes—that is to say, by the nature of the plant and the situation of the node upon it. This appears to be the case for all nodes of axillary branches in *Vicia faba*. By growing large numbers of seedlings and appropriately mutilating them it is possible to obtain grown shoots from almost any selected bud-position. A study of material grown in this way leads to the following empirical law, no exception to which has yet been observed:

> *At every node of an axillary shoot the buds which occupy the odd-numbered positions in the axillary series are placed on the side towards the parent shoot.*

This rule completely determines the state of any node of any axillary shoot (even of those arising in the axils of the cotyledons) and it implies, as shown in Fig. 2, that the nodes of any single shoot will be alternately R and L. Viewing any bud-series from the direction of the stem, buds in the right-hand half of the zigzag will have their odd-numbered nodes R, those in the left-hand half will have their odd-numbered nodes L.

As a basis for the development of a progressive morphology the study of axillary branches thus appears to be quite sterile, and it has not been pursued in the other species investigated.

Plumular shoots. The upper part of the plumule in *Vicia* resembles the axillary shoots in having a regular alternation of R and L nodes, all the odd-numbered members of the axillary bud-series being turned towards the same side of the foliar plane. Both in this region of the plumule and in the axillary branches the structure of the leaf is related to the disposition of its axillary buds. Whenever the number of leaflets to a leaf exceeds 2 the leaflets are placed alternately on the rachis, and the lowest (proximal) leaflet is on the same side as the odd-numbered buds. This relationship, to which the lowest multifoliolate leaves of a shoot sometimes fail to conform, is shown in Fig. 3, which is a diagrammatic plan of a shoot, two consecutive leaves and their buds being conventionally represented. In some instances the side of the leaf towards the odd-numbered buds actually has one leaflet more than the other.

The existence of a regular alternation of R and L nodes in the upper part of the plumular shoot (i.e. from about the 8th node upwards) clearly implies that there must be two types of seedling; that in which odd-numbered nodes are R and even-numbered ones L will be denoted by a −sign, a + seedling being one in which odd nodes are L and even ones R. Or, which amounts to the same thing, if, viewing the plumule from the side on which the cotyledons lie, odd-numbered buds are directed towards the right, then the plant is a — one, and vice versa. These relationships are shown diagrammatically in Fig. 4.

Whereas the origin of an axillary shoot is an inherently unsymmetrical process, so that its paramount influence in deciding the sign of the shoot's nodal alternation need occasion no surprise, the basic design of a bean seedling is perfectly symmetrical about the foliar plane, and there is no *a priori* reason why a plumule should be of one sign in preference to the other. A count of seedlings yielded the figures 226+, 238—. The difference is too small to encourage any hope that a statistically significant preponderance of one sign might be demonstrated with any reasonable number of observations. There is so far no evidence that the sign of a plumule is not a matter of pure accident.

ARRANGEMENT OF BUDS

It remains to consider the positions of the buds in the axils of the lower leaves and of the cotyledons. Usually about the first four leaves of a bean plumule (i.e. the two scale-leaves and the first two foliage-leaves) have well-developed axillary buds, and are followed by several leaves (generally about nodes 5 to 7 inclusive) which, under ordinary conditions, never develop detectable buds. Each cotyledon has a bud-series essentially similar to that of a leaf, but these buds occupy a special position since the cotyledons are not attached in the foliar plane of the plumule but roughly at right angles to it.

A cursory examination of the lower part of the plumule in *Vicia* shows that both R and L nodes occur, but that their distribution is extremely variable. Such a situation offers an opportunity for the investigation of some of the factors which may influence the development of the buds. The principal possibilities which have to be considered are:

(*a*) An inherent tendency for the species to produce more nodes of one type than of the other.
(*b*) A relationship between the position of a node on the plant and the probability that that node will be R.
(*c*) An interaction between nodes, the probability that a given node will be R depending on the state of other nodes of the same individual.

Interactions between nodes. The issues raised under (*a*) and (*b*) above must for the present remain in doubt. The observed frequencies of the R and L conditions at the first four nodes of the plumule in *Vicia faba* are given in Table I. These figures are insufficient to show that the probability of the R-condition is not exactly $\frac{1}{2}$ for every position on the plumule. It may be as well to state at this point that similarly inconclusive results have been obtained with all the other plants mentioned in this paper. The cotyledonary nodes in this respect as in others require separate consideration and will be dealt with later, but so far as the ordinary plumular nodes are concerned there seems to be little prospect of demonstrating any significant departure from randomness so long as the methods employed treat the state of each node as an isolated occurrence.

TABLE I

Frequency of Occurrence of R- and L-conditions at the First Four Plumular Nodes of Vicia faba

	R.	L.
1	265	270
2	281	253
3	239	264
4	89	109
Totals	874	896

If, on the other hand, the condition of each node is considered in relation to that of others, it can easily be shown that the two nodal states are *not* distributed at random. Considering the first three epicotyledonary nodes of *Vicia faba*, each of which may be either R or L, it is clear that eight different types of seedling ought to exist, one for each of the node-sequences set out in the first column of Table II, and that a completely random distribution would result in all eight types being equally common. In the second column of the table are shown the frequencies of occurrence of the eight combinations in a random sample of 500 seedlings, the expectation being 62·5 for each class. The relevant value of χ^2 is 26·27, which with seven degrees of freedom, corresponds to a probability of less than 0·001. By the ordinary standards of biological research such a level of significance is reasonably satisfactory, but one must not too hastily conclude that this result necessarily implies any interaction between nodes. Another hypothesis remains to be examined. Suppose that the slight inequalities in frequency of R and L nodes which appear in Table I, although not reaching an acceptable level of statistical significance, are genuine characteristics of the material. Then by postulating random association of nodes having the observed frequencies an alternative set of expectations can be calculated and entered in the third column of Table II.

TABLE II

Frequency of Occurrence of Different Combinations at the First Three Plumular Nodes of Vicia faba

	Frequency.	Expected frequency.
RRR	80	61·93
RRL	74	68·40
RLR	49	55·75
RLL	42	61·59
LRR	45	63·09
LRL	67	69·69
LLR	64	56·81
LLL	79	62·75

This hypothesis leads to a value of χ^2, still with seven degrees of freedom, of 23·19, corresponding to a probability rather greater than 0·001, though still much less than 0·01; the second hypothesis does not fit the observations very much better than did the first.

Geometrical relations. These calculations, moreover, are statistically inefficient because they ignore one of the geometrical regularities of the system. Obviously two seedlings with the node-sequences RRR and LLL have in common something which is not shared by such plants as RRL and LRL, and which is entirely independent of the distinction between R and L nodes.

TABLE III

Frequency of Occurrence of Different Rhythms at the First Three Plumular Nodes of Vicia faba

	Frequency.	Expected frequency.
AAA . . .	159	124·68
AAB . . .	138	125·21
ABA . . .	116	125·44
ABB . . .	87	124·68

We may express properties of this kind by substituting the letters A and B for R and L; A and B will then play much the same part as they do in the rhyming schemes used in the analysis of verse. With a succession of three nodes there are four alternative rhythms starting with A. In Table III the observed and expected frequencies are given, all the entries in this table being obtained by addition from those in Table II. χ^2 has the value 22·86, but has only three degrees of freedom and thus corresponds to a probability of about 0·0001. This higher level of significance is reached because we have now, for the first time, taken account of the marked resemblances between the top and bottom halves of the second column in Table II.

Similar calculations have been made for several other sets of observations, both on *Vicia faba* and on other species. The values of χ^2 obtained are uniformly high, sometimes far beyond the range of the standard tables. As more observations are taken into account the conjoint probability of the departures from expectation being due to sampling error rapidly drops to a level beyond which its further pursuit would appear to be supererogatory. It is for all practical purposes a certainty that the nodes of a shoot are never, within the taxonomic range of the present discussion, a random assortment.

It is impossible to avoid the conclusion that some kind of stimulus or impulse capable of influencing the arrangement of the buds must be transmitted from one node to another. Even in cases where R and L nodes occur in regular alternation there must be some means by which each node, at the instant when its symmetry is decided, can experience the influence of the older nodes lower down the stem. The physical nature of the effective agent, the manner of its transmission, and the exact location of the route along which it travels are matters which appear at present to be beyond the reach of anything but idle speculation. The immediate problem is to find a means of measuring the action of one node upon another and to determine, as far as the available data will allow, the quantitative laws which govern the propagation of this unknown influence.

Some qualitative indications of the relationships existing in the bean plumule can be extracted from the observations by the methods already introduced. Reference to Table III will show that the rhythms AAA and AAB are more common than ABA and ABB. That is to say, the second node tends to resemble the first. Further, AAA is more common than AAB and ABA is more common than ABB. That is to say, when the first two nodes agree the third tends also to conform, but when the first two nodes differ the third tends to continue the alternation. From Table IV, which shows the observed frequencies of all possible rhythms for the first four nodes of the bean plumule, it appears that the only sequence of the first three nodes which has any very marked influence on the state of the fourth is the alternating sequence ABA, and that this system operates on the fourth node in such a way as to encourage it to continue the alternation. It is as though an initial tendency for all nodes to be alike were being opposed and ultimately overcome by a tendency for the two forms to alternate.

TABLE IV

Frequency of Occurrence of Different Rhythms at the First Four Plumular Nodes of Vicia faba

	Frequency.
AAAA . . .	34
AAAB . . .	32
AABA . . .	28
AABB . . .	29
ABAA . . .	9
ABAB . . .	31
ABBA . . .	20
ABBB . . .	15

Unfortunately it is quite impracticable to carry out any extended investigation on these lines. As the number of nodes increases, the number of observations required increases exponentially. Furthermore, the method can only utilize an unbroken 'run' of R/L determinations for successive nodes of a shoot. As the number of nodes to be scrutinized on each specimen increases, one encounters a sharply rising wastage of effort owing to the occurrence here and there of nodes which, through accidental damage and other causes, cannot be classified. What is required is a method of extracting a numerical value for the influence of one node upon another irrespective of the state of the remainder of the shoot. Such a method would make the most effective use of the observations, since besides being able to utilize incomplete sets of observations it would also avail itself of every opportunity of pooling the data (it would, for instance, draw information about bean seedlings with the first node R and the fourth node L from all the types RRRL, RRLL, RLRL, and RLLL). It will be of little consequence if the system of calculation which is adopted is not always convenient for the evaluation of the statistical significance of particular results. Observations are available which, as shown by rigorous statistical tests, are amply sufficient to establish the existence of some interaction between nodes; in a first exploration of the subject it is reasonable to accept, subject to the exercise of ordinary caution, any indications which those observations can furnish as to the nature and extent of the interaction.

Quantitative treatment. After trying a number of alternative approaches to the problem, I propose the following treatment. It is to be supposed that in any one interaction only two nodes are involved. The lower and older node (to be called the *transmitter*) emits a signal which is characteristic of its condition and which ultimately arrives at the upper and younger node (the *receiver*), where it plays a part in deciding the symmetry of the bud-series. In order to obtain a measure of the effective signal-strength at the receiver, we extract from the data the observed frequencies of four classes of plants, as shown at the right of Table V, which is given as a specimen case. Taking the two frequencies for plants with R transmitters we divide the greater number by the lesser and enter the quotient in the table. A second quotient is obtained from the two frequencies of plants with L transmitters. Signs are then attached to the quotients according to the following rule:

TABLE V

Specimen Case to show how Transmission Values are calculated. (Nodes 3 and 8 of the plumule of Pisum sativum, '*Tall Sugar*')

State of node 3 (transmitter).	State of node 8 (receiver).	Number of plants in sample.	Calculation.	Transmission values.
R	R	20	35/20 = 1·750	**−1·750**
R	L	35		
L	R	18	22/18 = 1·222	+1·222
L	L	22		

> *A quotient is positive when a majority of observations show agreement of the receiver with the transmitter, negative when the dominant tendency is towards disagreement. A quotient which is equal to unity carries no sign.*

Finally the quotient which has the numerically greater value (signs being disregarded) is distinguished by being put in heavy type.

The paired quotients obtained in this way will be known as *transmission values.* From any given set of observations a *transmission table* can be prepared in which every possible combination of transmitter and receiver is represented by a pair of transmission values. For a sequence of n nodes there will be $n(n-1)$ transmission values, two for each of the $\frac{1}{2}n(n-1)$ cells in the transmission table.

The transmission table for the first four plumular nodes of *Vicia faba*, calculated from the same data as were used in previous working, is given as

Table VI. Transmission tables have also been calculated for the first six nodes of *Cicer arietinum* and for the first nine nodes of each of the three varieties of *Pisum*. It will be unnecessary to reproduce all these tables in detail, but that for 'Tall Sugar' pea is given (Table VII) as a more extensive example.

TABLE VI

Transmission Table for the First Four Plumular Nodes of Vicia faba

Serial numbers of transmitters.	Serial numbers of receivers 2	 3	 4
1	**+1·60** +1·26	+1·12 **+1·23**	**−1·43** +1·04
2		**−1·14** +1·08	−1·02 **+1·51**
3			**−1·58** −1·04

By a simple arithmetical device the complex problem of estimating nodal interactions has now been transformed into the much simpler one of studying the comparative morphology of transmission tables. It will be well to begin by translating into terms of transmission values the two hypotheses already considered and shown to be untenable. The first hypothesis (i.e. that the probability of the R-condition is ½ for all nodes, with no interaction) obviously leads to the expectation that all transmission values would be unity. The second hypothesis (that nodes are independent with probabilities differing from ½) is slightly more complicated. Let the probability of the R-condition

TABLE VII

Transmission Table for the First Nine Plumular Nodes of Pisum sativum, *'Tall Sugar'*

	2	3	4	5	6	7	8	9
1	**+1·30** −1·04	**+1·36** +1·13	+1·13 **+2·06**	**+1·46** +1·23	+1·46 **+1·50**	**−1·25** +1·24	**−1·57** +1·44	**−1·71** +1·27
2		**+1·42** +1·15	−1·13 **+1·43**	+1·03 **−1·36**	**+1·06** 1·00	+1·18 **+1·38**	**−1·57** +1·41	**−1·79** +1·19
3			−1·03 **+1·60**	**+1·18** −1·04	+1·07 **−1·44**	+1·31 **+1·35**	**−1·75** +1·22	−1·41 **+1·50**
4				**+1·24** −1·10	**+1·56** +1·21	**−1·67** −1·44	−1·32 **+1·33**	**−1·54** +1·47
5					−1·06 **−1·50**	−1·25 **−1·38**	**−1·63** +1·30	−1·12 **+1·69**
6						+1·08 +1·08	−1·40 **+2·00**	+1·13 **+1·43**
7							−1·50 **+1·68**	−1·19 **+1·50**
8								**−3·71** −3·31

be a for the transmitter and b for the receiver. Then the expected frequencies are:

Transmitter R, receiver R: ab
Transmitter R, receiver L: $a(1-b)$
Transmitter L, receiver R: $b(1-a)$
Transmitter L, receiver L: $(1-a)(1-b)$

The transmission-values would be $+b/(1-b)$
$-b/(1-b)$

when $b > \frac{1}{2}$, and $-(1-b)/b$
$+(1-b)/b$

when $b < \frac{1}{2}$. In either case the sum of the two transmission values of a pair would be zero.

As one might expect, it is impossible to find in any of the transmission tables any considerable area over which the prediction of either of these hypotheses is even approximately fulfilled. On the other hand, it is easy, with an adequate technique, to detect certain other regularities and to show that they run through the whole range of the observations on which this paper is based. A simple example will illustrate the procedure employed.

Consider the structure of the transmission table for a shoot in which there is a tendency for nodes to alternate according to the rhythm ABABABABABA . . . . Obviously any cell of the table for which transmitter and receiver are separated by an even number of internodes will tend to contain positive values, while if the distance is an odd number of internodes the transmission values will be predominantly negative. Let n_T be the serial number of the transmitter and n_R that of the receiver. Then any cell in the table affords positive evidence for the existence of an alternating tendency if the larger transmission value within it has a $+$ or $-$ sign according as $(n_R - n_T)$ is even or odd.

The distribution of such cells throughout the tables may be shown by shading the corresponding squares in an appropriate set of blank charts (Fig. 5, top row). It is clear at once that in *Cicer* the alternating tendency extends throughout the region investigated, while in *Vicia* it is only the relationship between nodes 1 and 2 which constitutes an exception to the alternating pattern. The significance of the diagrams for the three pea varieties is less immediately apparent, and a satisfactory interpretation can only be reached by carrying the analysis one stage farther.

Let us assign to each cell in the transmission tables a number which we may call its *altitude*, equal to $\frac{1}{2}(n_T + n_R)$. The mean altitude for all the cells of the table is given at the bottom of each column in Fig. 5. The number beside each chart is the mean altitude of the shaded squares only. The values in the top row indicate that (except of course in *Cicer*) the tendency to alternation is most noticeable in the upper parts of the plumules.

Another possible stable condition of a shoot is that in which all nodes tend to have the same form (the rhythm AAAAAAAA . . .). In such a case all transmission values would be positive, and evidence for the existence of such a tendency is offered by any cell in which the larger transmission value is positive. The appropriate set of charts is given in the second row of Fig. 5.

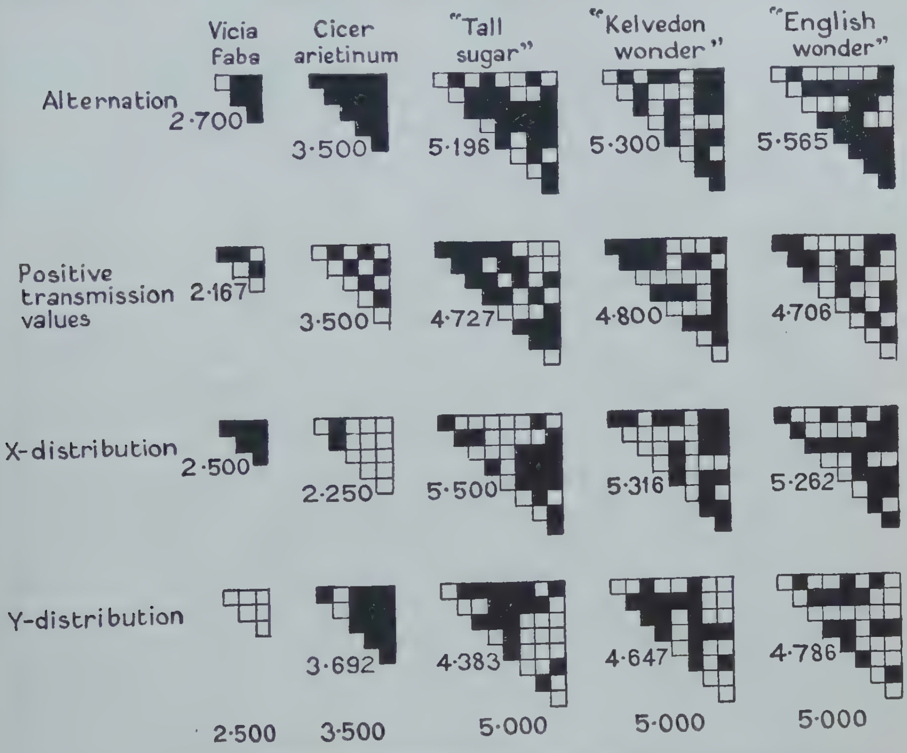

FIG. 5. Charts showing the distribution of various relationships in the transmission tables for *Vicia*, *Cicer*, and three varieties of *Pisum sativum*. The number at the foot of each column is the mean altitude for all the squares of the chart, the number beside each diagram being the mean altitude for shaded squares only.

The mean altitudes of the shaded squares indicate clearly that the tendency to produce an AAAAAAAA . . . sequence is found mainly in the lower parts of the plumules.

In the case of *Vicia faba* the analysis has so far done no more than restate in more precise terms the conclusion (already drawn from an inspection of Tables III and IV) that in the development of a plumule an alternating pattern is superimposed on an initial tendency for all nodes to be alike. The treatment in terms of transmission values has greatly facilitated the extension of this principle to the three pea varieties, and on these grounds alone its adoption would have been fully justified. But its principal merit lies in its power to detect relationships of a less obvious kind. The effects which are now to be discussed are of a completely novel character; by this I mean that, so far as can be ascertained, nobody has previously contemplated the possibility that such phenomena might exist.

If the relationship between the condition of one node and that of another is really due to the transmission of a stimulus in the manner postulated, it is necessary to suppose that the signal emitted by an R transmitter is in some way different from that which would have been sent out by an L transmitter in the same position. It follows that the tissues through which the signals are propagated will not necessarily be equally efficient conductors for both. The transmission hypothesis therefore leads to the expectation that the effective signal-strength at the receiver (which is what a transmission value is designed to measure) will depend in an orderly manner on whether the transmitter is R or L, the variation being due partly to a relationship between the state of a node and its power as a transmitter, partly to a selective or filtering action by the intervening tissues.

Possibility of regular patterns. The simplest conceivable phenomenon of this kind would be a tendency for the larger transmission value of each pair to be associated constantly with one condition of the transmitter rather than the other. An exhaustive examination of all the available data makes it practically certain that such a relationship does not exist in the material of the present investigation.

There is strong evidence, however, for the existence of another slightly more complicated system. Examine, for example, the transmission table for *Vicia faba* (Table VI). Here there is a regular alternation of squares in which the transmission value for R transmitters is the larger with others in which it is the lesser of the two. When $(n_R - n_T)$ is odd, the condition of the receiver is more affected by that of the transmitter if the latter is R, while if $(n_R - n_T)$ is even an L transmitter exerts the greater influence. Let us call this state of affairs an X-distribution and its converse a Y-distribution. The appropriate diagrams are given in Fig. 5 (third and fourth rows respectively). The evidence for the reality of the phenomenon may now be summarized thus:

(*a*) In the *Vicia* table all 6 cells conform to a single distribution.
(*b*) In the *Cicer* table 13 cells out of 15 conform to a single distribution.
(*c*) All three pea varieties agree in having one type of distribution characteristic of the lower, the other of the upper parts of the plumule.

A simple calculation shows that in drawing samples from a population of tables in which the position of the larger transmission value within a cell was independent of the position of that cell in the table, the odds against obtaining a sample showing this much bias (or more) would be, to the nearest whole number, 17,331 to 1. This figure represents a level of statistical significance which compares favourably with those commonly regarded as acceptable in more conventional investigations.

In *Vicia faba*, in recording the condition of the lower plumular nodes, a note was also kept of the sign of the regular alternation of the upper nodes. It is therefore possible to examine the relationship of the lower nodes not only to each other but also to the upper nodes at which the inflorescences are produced. Any lower node must either conform to, or conflict with, the ultimate stable alternation, and it is sufficient to determine the proportion of conforming nodes for each position on the lower part of the plumule. Expressed as percentages, the proportions for the first four epicotyledonary nodes are: 55·92, 59·21, 58·42, and 65·54. These figures are of interest principally as giving an independent confirmation of the conclusion already reached regarding the progressive development of an alternating tendency in the lower part of the plumule.

TABLE VIII

Frequency of Occurrence of Different Combinations at the Cotyledonary Nodes

	Vicia faba.	'Tall Sugar.'	'Kelvedon Wonder.'	'English Wonder.'
(RR)	47	11	2	1
(RL)	400	16	1	2
(LR)	29	38	101	112
(LL)	37	15	1	4

The buds in the axils of the cotyledons are essentially similar to those at other nodes, and their disposition may be denoted by the use of the letters R and L according to the convention already established, but in order to achieve a completely unambiguous description of a seedling it will be necessary to make an arbitrary distinction between the two cotyledons. Viewing a seedling from the side, with the cotyledons lying to the left of the main axis (as in Fig. 4) the cotyledon which is nearer to the observer will be treated as the 'first' cotyledon, the one behind it as the 'second'. As a safeguard against confusion, letters referring to cotyledonary nodes will be placed in brackets. The numbers of plants observed with different arrangements of cotyledonary buds are given in Table VIII, from which the following conclusions may be drawn:

(*a*) In *Vicia* there is a great excess of (RL) and in *Pisum* of (LR) plants.

(*b*) These patterns are symmetrical about the foliar plane, (RL) plants having the odd-numbered buds directed away from the first plumular leaf, (LR) plants towards it.

(*c*) In each of the four samples there is evidence that when one of the cotyledonary bud-series departs from the condition which is commonest for the species the probability that the other will also do so is greatly increased. That is to say, (LR) is commoner in *Vicia* and (RL) is commoner in *Pisum* than would have been expected if the conditions of the two bud-series were independent.

The *Vicia* observations produce a value of χ^2 of about 48, which, with one degree of freedom, corresponds to a probability of the order of 10^{-14}.

It is natural to ask whether there is any correlation between the state of the cotyledonary buds and that of those at higher nodes. Owing to the low frequency of occurrence of (RR) and (LL) seedlings it has not been possible to obtain statistically significant evidence on this point, but within the range of the observations it is noticeable that most (RR) seedlings have the first plumular node R, while in most (LL) plants it is L.

No information is available concerning the cotyledonary buds of *Cicer arietinum*, but the cotyledons themselves display an interesting anomaly which has no counterpart in the other species examined. Whereas in the Vicieae generally the two cotyledons, enclosed in the testa, lie on the side of the seedling remote from the first plumular leaf, in *Cicer* this is not always the case. In a sample of 50 seedlings there were 17 with the first plumular leaf directly above the 'seed', the other 33 being perfectly normal.

Discussion and Interpretation of Results

In plants, as in animals, there are many structural and functional relationships which possess the attributes of right- or left-handedness. In dealing with phenomena of this kind it is necessary to consider both the nature of the elementary stereoisometric configurations (e.g. the arrangement of buds at a node, the aestivation of a corolla, or the helix in a spiral xylem element) and the manner in which the elementary configurations are distributed in a population. According to the manner of their distribution, elementary configurations can be divided into two great classes, distinguished by a criterion which is most conveniently stated in terms of optical reflection.

Let any real population be completely described in respect of a given elementary configuration. Then suppose the population of plants to be placed before a plane mirror, and prepare a corresponding description of the population of virtual images. Either there will be a statistically significant difference between the two descriptions, in which case the elementary configurations are so distributed as to produce a general asymmetry of the population, or there will be no significant difference, suggesting that in spite of the asymmetry of its parts the population as a whole is a symmetrical entity in respect of the particular elementary configuration involved.

When a general asymmetry of the population can be demonstrated the only possible explanation is some kind of genetical control. In a perfectly symmetrical population the only genetical control which can exist is some kind of balanced system analogous to that which, in some organisms, ensures that the two sexes shall occur in equal numbers. Symmetry being, from the statistical point of view, a negative quality, a belief in its existence can only be based on a lack of evidence of asymmetry; nevertheless if an extensive investigation fails to reveal any general asymmetry in a population there is a strong presumption that the elementary configuration in question is not subject to direct genetical control.

The only considerable group of stereoisometric configurations which do not produce any general asymmetry of plant populations are phyllotactic spirals and various floral phenomena ultimately referable to a phyllotactic origin. With only trivial exceptions, all other right- or left-handed relationships, from corolla-aestivation to the overlap of the leaf-sheaths in grasses and the spiral coiling of liverwort elaters, are associated with conspicuous asymmetry of the population, and are clearly subject to genetical (though perhaps not genic) control. Except in some Scitamineae, on the other hand, all attempts to demonstrate an asymmetry of a population in respect of phyllotactic spirals have failed.

There would be no point in reproducing here the details of the various tests for population-asymmetry which have been applied to the available data on axillary bud-series, since the results have been consistently negative. Except for the relationships involved in the X- and Y-distributions the populations examined must be supposed to be completely symmetrical. A single example will serve to illustrate the nature of this symmetry. An RLL bean plumule, placed before a mirror, produces a reflection LRR. In a sample of 500 plants the sequence RLL occurred 42 times. The frequency of the sequence LRR in the sample of 500 reflections is therefore 42. But in the sample of real plants there were 45 cases of LRR, and the difference is not significant. Similarly with other combinations, as also with more complex classifications taking into account the cotyledonary bud-series and the + and — alternations. There is thus a strong presumption that the state of a node is independent of any direct genetical control and that the agencies which influence it resemble those involved in phyllotactic relationships rather than, for example, the hereditary protoplasmic anisotropy which determines the sense of the helices in the protoxylem.

If the reality of the X- and Y- distributions be taken as established (and by the ordinary standards of biological research the evidence is extremely strong), some important conclusions follow. It is impossible to explain these distributions by any hypothesis which merely attributes particular qualities to transmitters or receivers. The characteristics which have to be accounted for are not characteristics of nodes but of the routes between them. If a direct route is one connecting leaves on the same side of the stem, while an oblique route connects leaves on opposite sides of the stem, an X-distribution will result only if direct routes transmit L signals more effectively than R signals, while oblique routes favour the propagation of the latter. A reversal of these preferences will bring about a Y-distribution. An acceptance of the reality of these distributions automatically involves the conclusion that the tissues of the plant may act as a filter, passing one kind of bud-determining influence more readily than the other, that direct and oblique routes behave differently, and that these filtering properties are genetically determined.

Two further questions arise naturally from the discovery of the interaction between nodes. Firstly, what is the law governing the addition of two impulses, derived from two transmitters, arriving either concurrently or successively at the same receiver? Secondly, does a signal from a transmitter to a distant receiver merely pass through intervening nodes or is it attenuated or amplified (or perhaps actively *relayed*?) by them? While I would be reluctant to regard these problems as insoluble, it is clear that they are not accessible to direct investigation by the methods so far employed, as it is impossible to produce plants with selected nodes omitted.

Some of the results of this work reveal considerable differences between the species used. Perhaps the most striking point is the fact that the arrangement of cotyledonary buds which is commonest in *Pisum* is precisely that which is least common in *Vicia faba*. 'Tall Sugar' is much less standardized as regards its cotyledonary buds than are the other two pea varieties; otherwise there is little evidence of intervarietal differences. Most of the observations appear from the taxonomic standpoint as unaccountable idiosyncrasies of the material which cannot be fitted into any coherent scheme at present, but the anomaly of seedling structure noted in *Cicer* does admit of a tentative phylogenetic interpretation. In a straight embryo with epigeal cotyledons the first leaf may arise indifferently on either side of the cotyledonary plane—in fact the two sides are usually indistinguishable. The regular plan found in most of the Vicieae represents a standardization appropriate to a curved embryo, and particularly to one with hypogeal cotyledons. The fact that this standardization is imperfect in *Cicer* can be related to the many other indications that this genus is the least specialized of its tribe.

SUMMARY

1. In *Vicia faba*, *Pisum sativum*, and *Cicer arietinum* each node produces a zigzag series of axillary buds. The series is unsymmetrical, so that right-handed (R) and left-handed (L) nodes can be distinguished.

2. On axillary shoots of *Vicia* R and L nodes occur alternately and the state of a node is determined entirely by its position.

3. On the upper part of a plumule of *Vicia* R and L nodes alternate regularly, but the apportionment of the R and L conditions to odd- and even-numbered nodes is determined by pure chance.

4. On the lower parts of plumules in all three species R and L nodes occur in various combinations and the state of any node is influenced by that of the nodes below.

5. In the early development of the plumule a tendency for all nodes to be alike is superseded by a tendency for the two types to alternate.

6. The influence exerted by one node upon the state of another is due to the transmission of an unknown internal stimulus. By conveying right- and left-handed impulses with different degrees of efficiency the tissues of the stem exert a filtering action upon this system of communication. The filtering properties are hereditary characteristics of the material.

7. The axillary buds of the cotyledons form various patterns depending on the genetical constitution of the material and undergo mutual interaction like that affecting other buds.

8. The condition of any individual node is probably not subject to direct genetical control. The processes which determine it are more akin to those involved in phyllotactic relationships than to most other right- or left-handed phenomena in plants.

9. The position of the plumular leaves relative to the cotyledons in *Cicer* is inconstant, suggesting descent from an ancestor with epigeal cotyledons.

Physiological and Ecological Studies in the Analysis of Plant Environment

IX. Adaptive Changes in the Vegetative Growth and Development of *Helianthus annuus* induced by an Alteration in Light Level

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With thirteen Figures in the Text

ABSTRACT

Pot experiments were carried out in which sunflowers in the early vegetative phase were first grown for a period under three levels of light (1·0, 0·5, and 0·24 daylight). Subsequently pots from each light group were subdivided into three so that in a second period plants could be subjected to the nine possible combinations of the same three light intensities before and after transference.

During the post-transference period of adaptation to either a higher or a lower intensity the net assimilation rate is logarithmically proportional to the light received and there is no residual effect of the initial light treatments. Eight days after transference the leaf-area ratios (total leaf area/total plant weight) at each light level become adjusted to a new equilibrium irrespective of the large initial differences in the ratio induced by the pre-transference intensities. In both periods there is an inverse and logarithmic relationship between the leaf-area ratio and falling light intensity; consequently, the greater the degree of shading in the pre-transference period, the higher are the mean ratios in the second period. Since the relative growth rate is the product of the net assimilation rate and the leaf-area ratio, the variations in the leaf-area ratio in the post-transference period induced by the initial light treatments are reflected in the relative growth rates. Thus plants transferred from a lower to a higher light intensity are leafier and initially grow faster than plants maintained at the higher level in both periods: the converse conditions lead to a reduction in the growth rate.

Shading depresses the growth of the roots, but the relative growth rate is dependent on the light intensity in both the pre-transference and post-transference periods. With transference from daylight to 0·24 daylight, the roots during the period of adjustment may lose weight, while the growth rate is maximal when plants are moved from the lowest to the highest intensity. In two out of the three experiments the relative growth rate of the shoot in the post-transference period is of the same order at all light intensities and is largely independent of the light received in the initial period.

In terms of leaf weight, decreasing the light intensity decreases the relative growth rate and there is no consistent after-effect of the initial light treatments.

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The rate of expansion in leaf area tends to be highest at the intermediate level of 0·5 daylight and over all the post-transference intensities the rates are maximal for those plants which received initially full daylight.

The ratio of leaf area to leaf weight is inversely and logarithmically proportional to the light level. After transference the slopes of the regressions are independent of the initial light treatments, but the mean ratios are inversely correlated with the initial degree of shading.

These adaptive changes to a variation in the light level are discussed with particular reference to the control of growth exerted by growth-regulating substances. It is concluded that on the basis of existing knowledge no adequate interpretation is yet possible.

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INTRODUCTION

IN the previous analysis of the factors governing the distribution of *Scilla non-scripta* in woodland it has already been shown that both the mean light intensity and the change in light intensity between March and June will operate in controlling the growth made during the season (Blackman and Rutter, 1946 and 1947). Under the conditions of deciduous woodland there is first an initial high light phase and subsequently, as the leaves of the canopy expand, there is a rapid increase in shading; in contrast, where the dominant trees are evergreen there is no such rapid change in the intensity. It was demonstrated experimentally (Blackman and Rutter, 1948) that when plants of *S. non-scripta*, which had been grown initially in full daylight, were transferred to shade conditions then their subsequent rate of growth was slower than plants which had been subjected to continuous shade in both periods. By the techniques of growth analysis it was further demonstrated that in the second period there was no difference between the two series in the net assimilation rates, but that as a result of the initial exposure to full daylight the leaf-area ratio was reduced and in consequence the relative growth rate depressed.

The original investigation was not extended to cover the reverse conditions, that is, the effects of a change in light intensity from a low to a high level. Such conditions arise in woodland when the dominant trees are coppiced or felled. Again, the circumstances will be similar, when a cover crop is removed or plants are transferred from a glasshouse to the open. Considering, therefore, both the ecological and agronomic implications it is surprising that so little attention has been paid to this aspect of light as an environmental factor. It is true that many papers have included relevant observations, but no reference has been found in the literature to precise experiments on the effects of transference from one light level to another.

The present investigation has aimed at an amplification of the results already obtained with *S. non-scripta*. The experimental procedure has been to subject batches of plants grown in pots to initial light intensities of 1·00, 0·50, and 0·24 daylight and subsequently to subdivide each light group into three and redistribute the sub-groups of pots between the three levels of light in the nine possible permutations. In this way the effects of a transference from one light level to either a lower or higher intensity can be investigated simultaneously. At the beginning and end of the first period and again at the end of the second period the appropriate number of pots were withdrawn and the dry weight of the root, stem, and leaves together with the leaf areas determined. From the resulting data the effects of the light treatments on net assimilation rate, leaf-area ratio, and the relative growth rate of either the whole plant or its constituent parts could all be determined.

The experiments were carried out on *Helianthus annuus*, for which the effects of shading on net assimilation rate and relative growth rate had already been established with precision (Blackman and Wilson, 1951*a* and *b*). Moreover, since the sunflower is an annual with a high rate of growth it seemed likely that its reactions to a change in the level of light intensity would be more sensitive than those of the slow growing *S. non-scripta*, in which the leaf primordia are laid down in the previous autumn.

EXPERIMENTAL METHODS

The experimental methods and techniques employed for these experiments were essentially the same as those used in previous investigations, and as they have already been described in detail (Blackman and Wilson, 1951*a*) only the main features will be recapitulated.

Glazed earthenware pots of 10 in. diameter and with a basal drain hole at the side were filled with a mixture of soil and sand to which a complete fertilizer had been added. Seed of the variety 'Pole Star' was then sown and after emergence the number of plants was reduced to 5. All pots received full daylight until the plants reached a standard size when they were matched by eye into groups of 3 or more pots—the number depending on the frequency of sampling—and each group, in four to sixfold replication, assigned at random to a light treatment.

At the initial and subsequent sampling occasions in each pot the shoots were cut off at ground level and the roots recovered by washing the soil through a series of sieves. Before the shoots, along with the roots, were dried at 100° C. and weighed, the leaf lamina were separated from the stem plus petiole and a sub-sample of the fresh leaves taken for the estimation of leaf area. These determinations were made by first recording the leaf outlines on a sheet of blueprint paper and then measuring the areas by means of a planimeter.

The method of reducing the light intensity was to suspend immediately above each block of pots small wooden frames covered with zinc sheeting perforated with a varying number and size of hole. Screens of this type ensured that there was a reduction in light intensity without a change in light quality. Moreover, by direct measurement it had already been shown (Blackman and Wilson, 1951*a*) that owing to diffusion and convection currents the air temperatures under such screens do not differ appreciably from those outside. The degree of shading was measured by means of a pair of photo-electric cells coupled to an ammeter with a two-way switch, so that almost simultaneous readings could be obtained from one cell in the open and the other placed beneath the screen.

EXPERIMENTAL RESULTS

I. *The effects of changes in light intensity on the leaf-area ratio*

The earlier experiments (Blackman and Wilson, 1951*b*) demonstrated that when young sunflower plants, raised in the open, are subsequently shaded there is a considerable change in the leaf-area ratio (total leaf area/total plant weight). The magnitude of the change and the final ratio reached is largely determined by the degree of shading, and it has been established both for *H. annuus* and other species that over the range of 0·2–1·0 daylight the mean leaf ratios during or after the period of adjustment bear an approximately logarithmic relationship to the light intensity.

On the basis of these findings it would be anticipated that when plants in the early vegetative phase are transferred to a higher or lower light level a process of readjustment will come into play and that ultimately new equilibria between the leaf-area ratio and the light level will be set up. In order, therefore, to assess the changes taking place during readjustment the time interval between sampling occasions needs to be varied. In the present investigation the period of the initial or pre-transference phase was kept constant at 8 days while the length of the 'post-transference' period varied from 8 days in expts. 31 and 32 to 4 days in expt. 33.

The changes in the leaf-area ratio between the beginning and the end of the post-transference period can be seen in Fig. 1. Firstly, in expts. 31 and 32 it is evident that by the end of 8 days the ratios at each light level have become adjusted to new equilibria irrespective of the large initial differences in the ratios which had resulted from exposure to the pre-transference intensities. In expt. 33 there is the same general trend, but because of the shorter interval between sampling occasions the adjustment is incomplete.

Since the experiments were so designed that some of the plants were grown under the same level of shading both in the pre-transference and post-transference periods it would be expected that under such conditions these

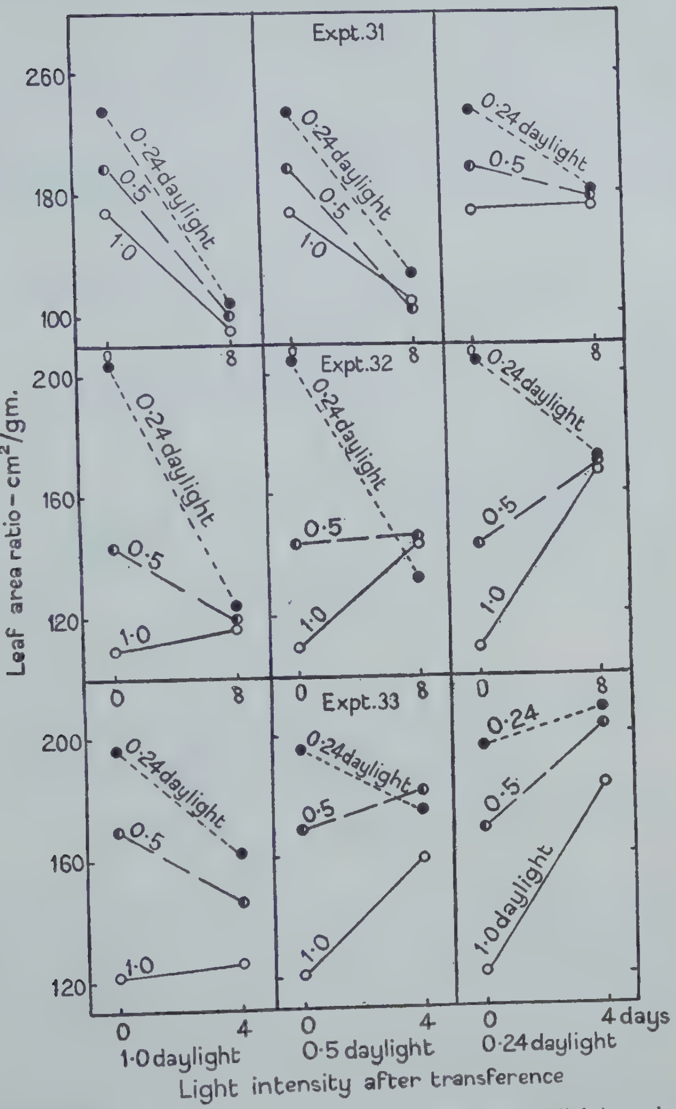

FIG. 1. The effects of varying light intensity (1·0, 0·5, and 0·24 daylight) on the changes in the leaf-area ratio between the beginning and end of the post-transference period of plants which before transference had been subjected to daylight (continuous lines), 0·5 daylight (broken lines), and 0·24 daylight (dotted lines).

ratios would show the smallest changes in the second period. Again the variation will be least when the experimental period is short (expt. 33) since it has already been demonstrated that there is an ontogenetic drift in the leaf-area ratio, i.e. the ratio falls with increase in size (Blackman and Wilson, 1951*b*). Thus the tendency in expt. 31 for the ratios to decrease during the post-transference phase can in part be ascribed to such a drift, because the relative growth rate, particularly in full daylight, was especially high with the result that the plants more than tripled their weight.

The data can also be examined from the viewpoint of the effect of the light levels in the pre- and post-transference periods on the mean ratios in the second period. In Fig. 2 for each experiment the mean ratios in the post-transference period have been plotted against the logarithm of the light intensity. The results are in very close agreement. The greater the degree of shading in the pre-transference period the greater are the leaf-area ratios in the second period. Irrespective of the level of light before transference there is a high correlation between the leaf-area ratio and the logarithm of the post-transference light intensity. In expt. 33 (Fig. 2*c*) statistical analysis showed that there was a small but significant departure from a linear relationship, but this was not so in the other experiments. Again, the regression lines in each experiment must be considered as parallel since the differences in slope are not significant.

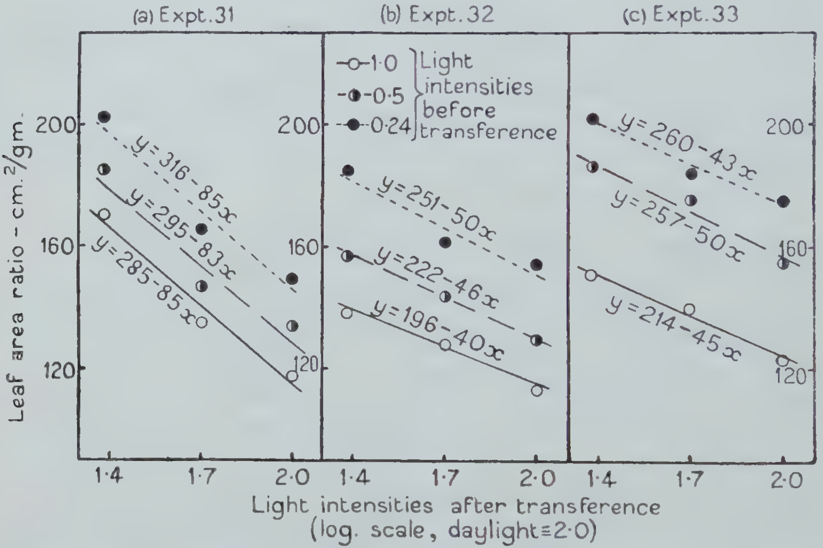

FIG. 2. The effects of varying light intensity on the leaf-area ratio of plants previously grown under a range of light intensities.

II. *The effects of changes in the light intensity on the net assimilation rate*

The combined influence of light intensity both before and after transference on the net assimilation rate is seen in Fig. 3. In each experiment the residual effects of light level in the pre-transference period are very small. In expts. 32 and 33 there is no suggestion that the net assimilation rate in the second period is influenced by the light level in the initial period; nor are there any significant differences in the slopes of the regression lines linking the assimilation rate with the logarithm of light intensity. However, in expt. 31

there is some indication that the plants grown in full daylight in the first period react somewhat differently in the post-transference period: for a combination of daylight in both periods the net assimilation rate is significantly greater than that of plants subjected to 0·24 daylight followed by 1·0 daylight. Again the compensation point—admittedly obtained by extrapolation—is greater for plants which were initially not shaded. On the other hand, at the intermediate intensity in the final period the residual effects of the initial light treatments are not significant. Thus, taking the results of expt. 31 as a whole, the evidence is not consistent that the after effects of the original light intensities are negligible. On the other hand, it is significant that in expt. 33, where because of the shortness of the second period any residual influence should be maximal, none is evident.

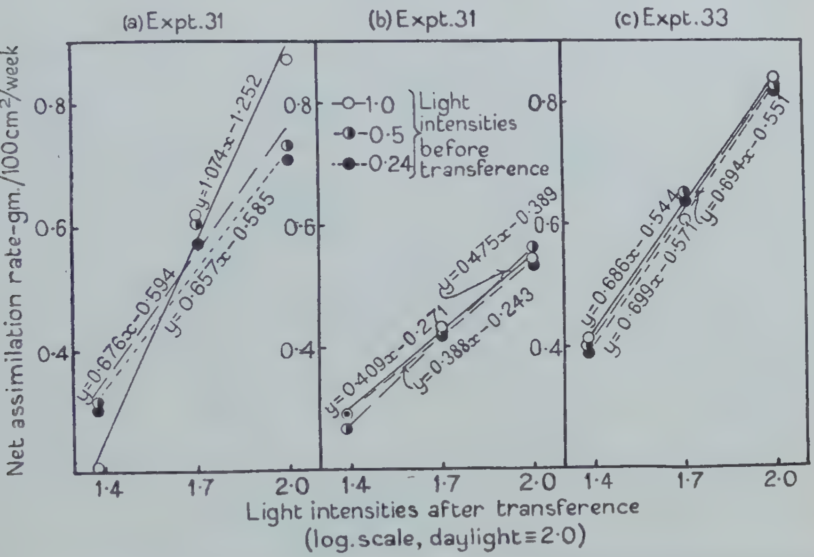

FIG. 3. The effects of varying light intensity on the net assimilation rate of plants previously grown under a range of light intensities.

The data in each experiment do emphasize the dominance of the actual light received in determining the rate of net assimilation. Quantitatively there is a close linear relationship between net assimilation and the logarithm of light intensity.

III. *The effects of changes in the light intensity on the relative growth rate*

Provided that the changes in plant weight are proportional to changes in the leaf area, it can be shown mathematically that the relative growth rate is the product of the net assimilation rate and the leaf-area ratio (see Blackman and Wilson, 1951*b*). Because of this interdependence it follows that the changes in the relative growth rate induced by transference from one light level to another will be governed by the corresponding changes in net assimilation rate and leaf-area ratio. It has already been demonstrated in Fig. 3 that in the post-transference period the net assimilation rate is little affected by the light level in the preceding period. Therefore the changes in relative growth rate after transference will be directly linked with the variations in the leaf-area ratio arising from the initial light treatments.

As plants moved from a lower to a higher intensity have a greater mean leaf-area ratio than those maintained in both periods at the higher level, it would be expected that they should grow faster. Conversely, plants transferred from full daylight to shade conditions should grow at a slower rate than the continuously shaded plants. These expected trends are found in all three experiments (Fig. 4), more particularly in expt. 33 (Fig. 4*c*), where because of the shortness of the post-transference period the residual influence of the pre-transference intensities is maximal.

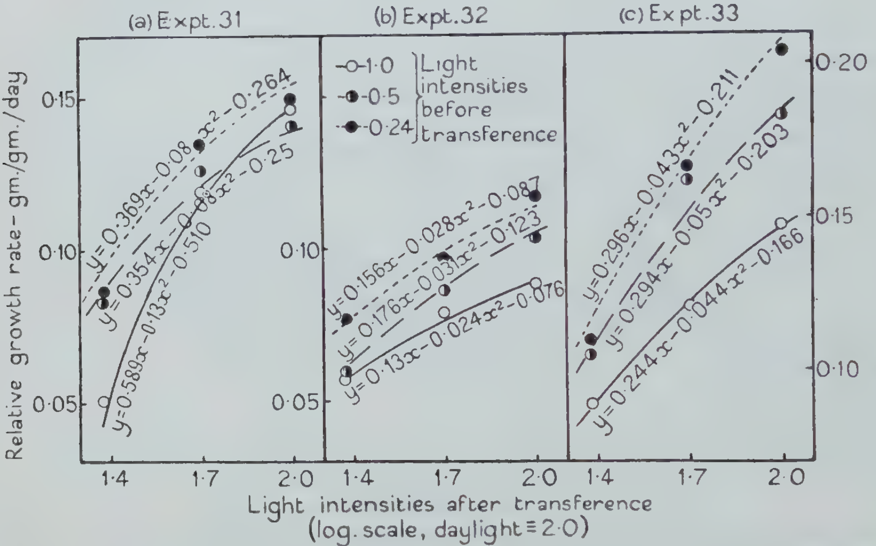

FIG. 4. The effects of varying light intensity on the relative growth rate of plants previously grown under a range of light intensities.

Since for both net assimilation rate and leaf-area ratio the relationships with the logarithm of light intensity can be expressed in terms of linear regressions it follows that the dependence of the relative growth rate on the light level can be expressed as the product of the two linear regressions. From Fig. 4 it is evident that the resultant curvilinear equations for relative growth rate are in reasonable agreement with the observed data.

IV. *The effects of changes in the light intensity on the growth of root, stem, and leaf*

It has been previously stated (p. 73) that at each sampling occasion the individual plants were divided into root, stem, and leaf, and it is therefore possible to assess the effects of the various light treatments on their growth. As the influence of shading on the comparative changes in the component parts of several species will be discussed fully in a subsequent paper, the present results will be largely considered from the aspect of the changes arising from transference from one light intensity to another. For this purpose two criteria have been selected. Firstly, the relative growth rates over the post-transference period have been calculated in the usual way for the individual parts. Secondly, in order to bring out possible differential effects on the growth of the three parts the changes in weight of the components have been expressed as proportions of the changes in the whole plant; they have been termed relative root, stem, and leaf growth.

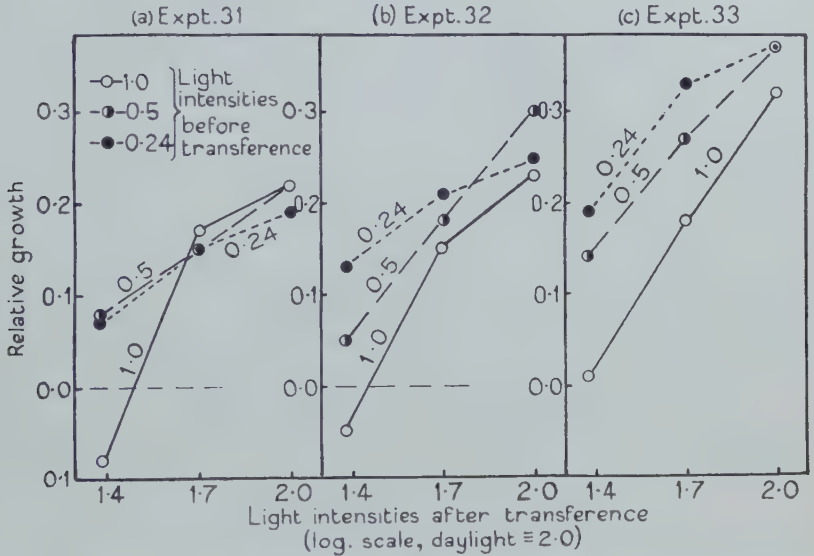

FIG. 5. The effects of varying light intensity on the relative root growth of plants previously grown under a range of light intensities.

(*a*) *Variations in root growth.* In each of the experiments the general effect of decreasing light intensity in the post-transference period is to depress relative root growth (Fig. 5). Superimposed on this depressant trend there is a marked residual influence of the initial light treatments. In expts. 31 and 32 plants subjected at first to full daylight make significantly less relative root growth than plants receiving in the first period 0·5 or 0·24 daylight. Indeed, transference from daylight to 0·24 daylight results in a negative relative root growth (expts. 31 and 32) or negligible growth (expt. 33).

These changes in relative root growth are closely paralleled by the changes in the relative growth rates of the roots. From Fig. 6 it is evident the growth rate is consistently diminished by shading in the post-transference period. The residual influence of the initial degree of shading is also apparent. There are two opposing trends. Transference from a lower to a higher light level accelerates the growth rate while the converse treatment depresses it. Thus a combination of 0·24 daylight and daylight leads to the maximum root growth while a reversal of the combination brings about in expts. 31 and 32 an actual loss in the root weight during the post-transference period.

(*b*) *Variations in stem growth.* During the post-transference period decreasing the light intensity increases the relative stem growth, irrespective of the initial light treatment—see Fig. 7. In expt. 31 relative stem growth is greatest when plants previously subjected to daylight are transferred to 0·24 daylight, but this trend is not significant in the other two experiments. From Fig. 8 it is apparent that in expts. 32 and 33 the relative growth rate is little affected either by the pre- or post-transference treatment. On the other hand, in expt. 31 a change from 0·5 to 0·24 daylight in the post-transference period causes a marked reduction in the growth rate. It has been already stressed (pp. 75–76) that in this experiment a large amount of growth was made in the post-transference period and this will tend to mask any residual effects of the pre-transference treatments. At the final sampling occasion the differences in the ratio of the stem to total plant weight which had been brought about by the pre-transference intensities had largely disappeared while this was not so in expts. 32 and 33. Thus the results of expt. 31 reflect the influence of light intensity after, rather than during, a period of readjustment.

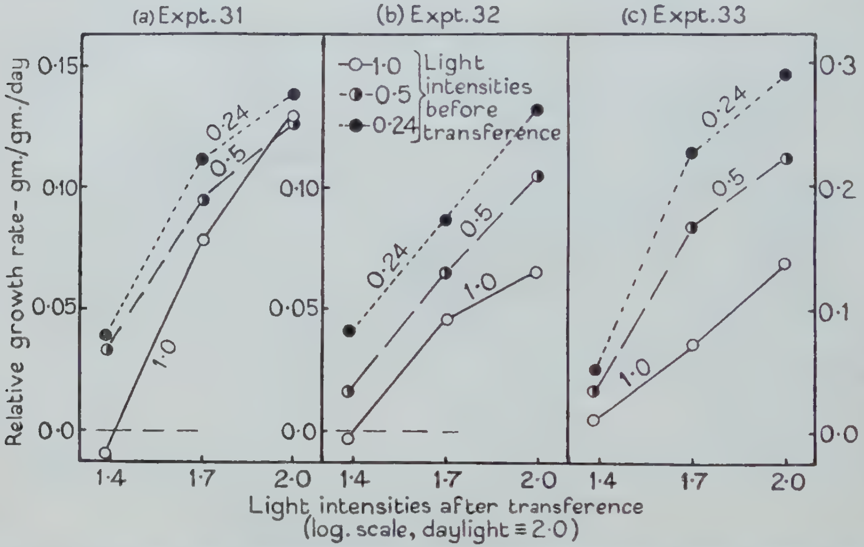

FIG. 6. The effects of varying light intensity on the relative growth rate of the roots of plants previously grown under a range of light intensities.

(*c*) *Variations in leaf growth.* In expt. 31 this same masking effect is also apparent in the results for relative leaf growth. It is clear from Fig. 9 that in each of the experiments there is no significant effect of varying light level in the post-transference period, but in expts. 32 and 33 there is a significant

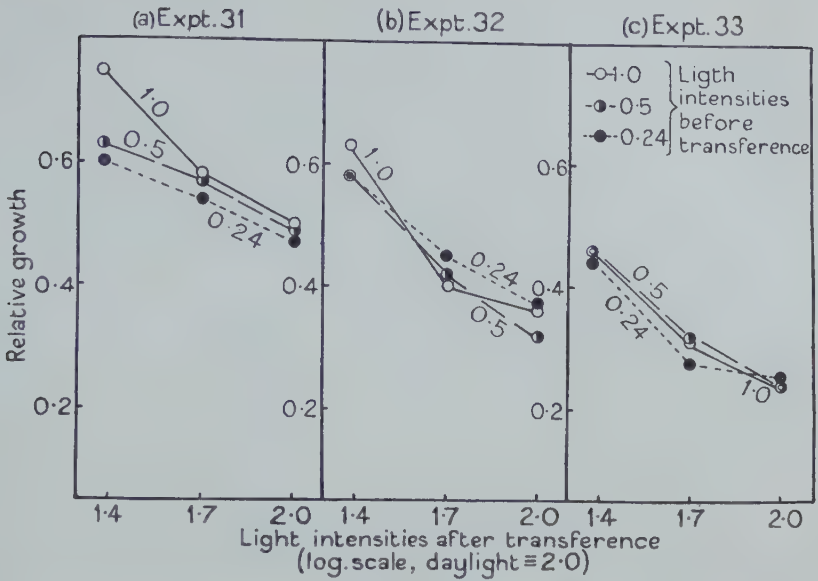

FIG. 7. The effects of varying light intensity on the relative stem growth of plants previously grown under a range of light intensities.

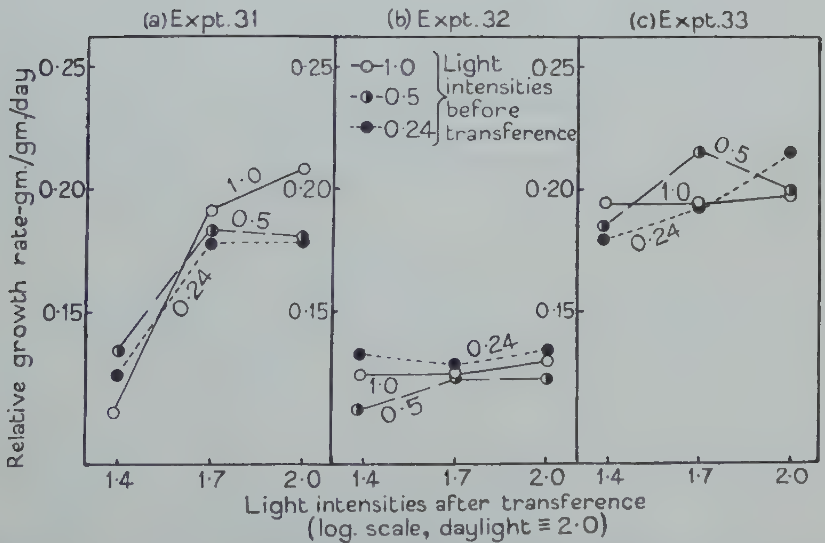

FIG. 8. The effects of varying light intensity on the relative growth rate of the stems of plants previously grown under a range of light intensities.

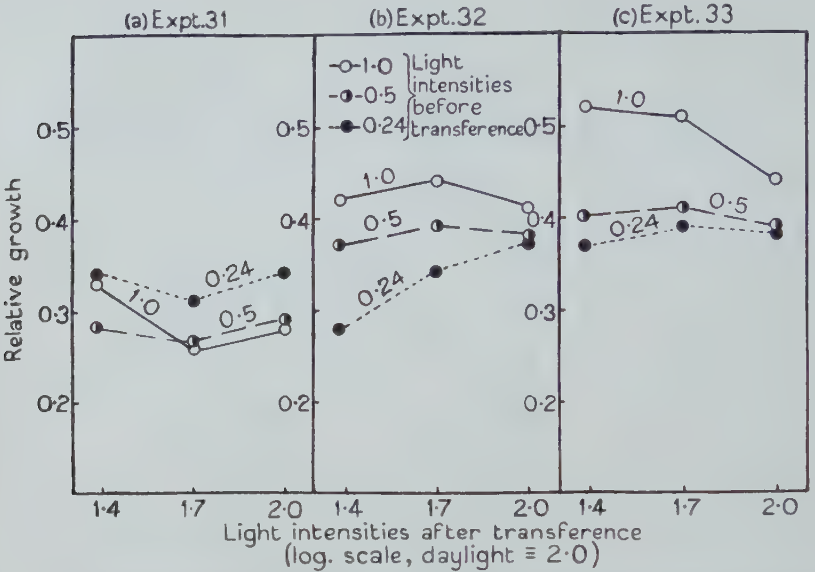

FIG. 9. The effects of varying light intensity on the relative leaf growth of plants previously grown under a range of light intensities.

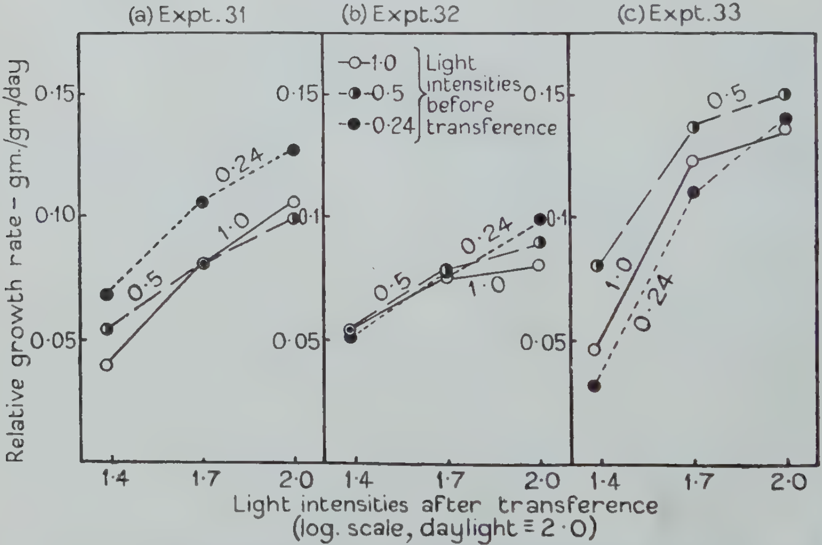

FIG. 10. The effects of varying light intensity on the relative growth rate of the leaves of plants previously grown under a range of light intensities.

residual influence of the pre-transference treatments. Plants initially grown in full daylight have subsequently a higher relative leaf growth.

Inspection of Fig. 10 shows that the relative growth rate of the leaves is largely controlled by the intensities in the post-transference period. In each experiment shading depresses the growth rate. There is no consistent common trend for the residual influence of the initial light treatments.

(*d*) *Changes in the leaf area per unit leaf weight.* It has been demonstrated in Fig. 2 that the leaf-area ratio increases logarithmically with falling light intensity, while it is evident from Figs. 9 and 10 that both the relative growth and the relative growth rate of the leaves are diminished by shading. It would therefore be anticipated that the ratio of leaf area to leaf weight should rise as the light intensity decreases. From Fig. 11 it is evident that this ratio is linearly related to the logarithm of the light intensity in the post-transference period. In none of the experiments do the differences in slope of the regressions for the pre-transference intensities reach significance. Initial shading has only a slight residual effect on the level of the ratios in expts. 31 and 32. But in expt. 33, where the second experimental period was so short that the modification in leaf structure was restricted, then the influence of the pre-transference light treatments was considerable. Leaves originally exposed to full daylight have subsequently at all light levels the smallest leaf area per unit leaf weight.

(*e*) *Changes in the total leaf area.* Since shading exerts opposite effects on leaf weight and on the leaf area to weight ratio, the influence of light intensity on the total leaf area will be dependent on the balance of the two opposing trends. From Fig. 12 it is evident that the trends roughly cancel out, for the relative growth rates on an area basis do not vary greatly with the level of light in the post-transference period. In all three experiments plants initially grown in full daylight have the highest rate of leaf expansion in the post-transference period and there is some suggestion that rate of change in leaf area is maximal in 0·5 daylight.

DISCUSSION

These experiments have demonstrated that when plants are transferred from one light level to another the changes taking place during the process of readjustment will be dependent upon whether transference has involved a change to a lower or a higher light intensity. This interdependence holds for the changes in the growth rates of the whole plant and its component parts but, in this respect, net assimilation rate is exceptional. It is evident from Fig. 3 that the residual effects of the initial intensities are small and the net assimilation rate is dependent on the light received in the second period. From Fig. 11 it is apparent, more particularly in expt. 33, that differences in the leaf-area to leaf-weight ratios caused by the initial light treatments still persist. From these two facts the conclusion could be reached that assimilation is independent of leaf structure since plants possessing leaves with a high ratio (shade leaf structure) fix carbon at the same rate as plants with a lower

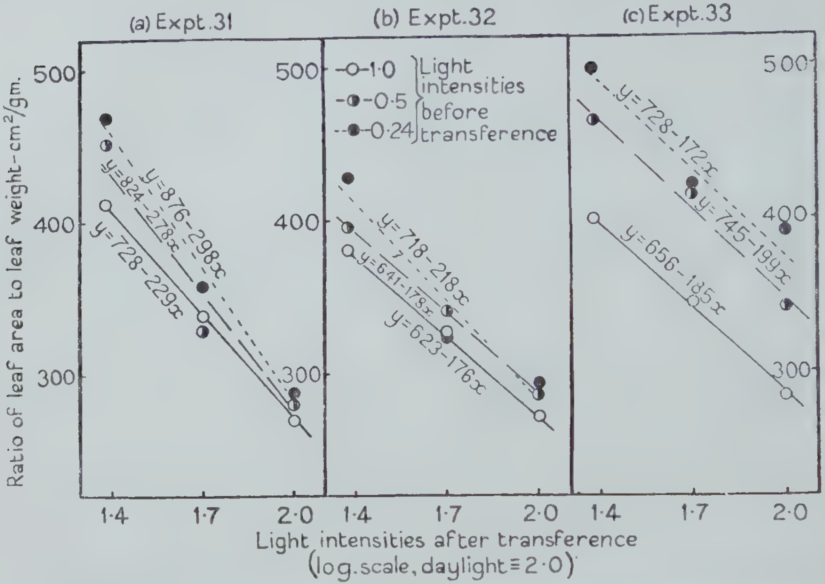

FIG. 11. The effects of varying light intensity on the ratio of leaf area to leaf weight of plants previously grown under a range of light intensities.

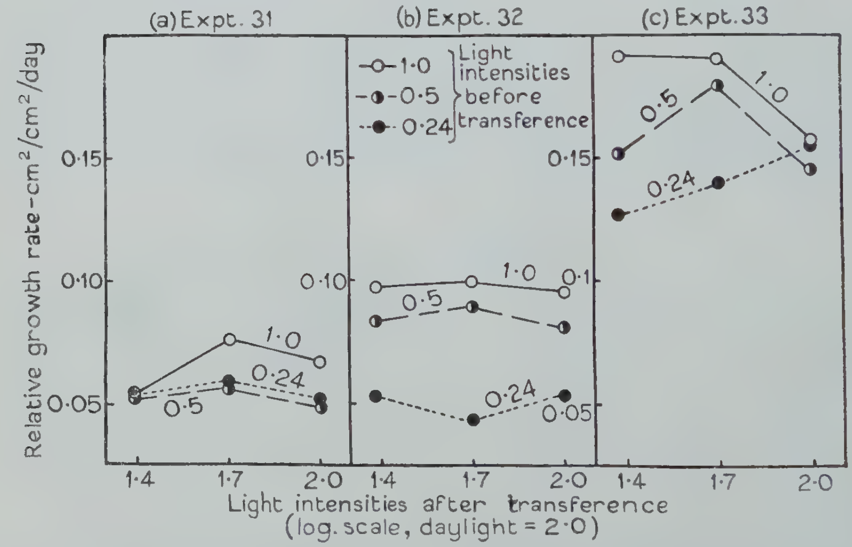

FIG. 12. The effects of varying light intensity on the relative growth rate in area of the leaves of plants previously grown under a range of light intensities.

ratio (sun leaves). This conclusion will only be valid if the diurnal respiratory losses of the whole plant have not been modified by the light treatments in the initial period. From the experimental results it is clear that the proportion of root, stem, and leaf is dependent on both the pre-transference and post-transference intensities and the general evidence, which has been reviewed by Blackman and Wilson (1951*a*), suggests that respiration is highest in roots. In parenthesis, it is emphasized that the evidence is indirect and inadequate since the respiratory losses in the different parts of intact plants, subjected to a diurnal cycle of light and dark periods, have not been directly assessed.

TABLE I

The Effects of Light Intensity before and after Transference on the Mean Relative Proportions of Root, Stem, and Leaf in the Post-transference Period

	Mean proportion—dry weight basis. Post-transference intensity.					
Pre-transference intensity.	Expt. 32			Expt. 33		
	1·0	0·5	0·24	1·0	0·5	0·24
Root						
1·0 daylight . . .	0·29	0·26	0·22	0·35	0·30	0·27
0·5 ,, . . .	0·29	0·24	0·21	0·33	0·28	0·24
0·24 ,, . . .	0·22	0·20	0·17	0·30	0·27	0·20
Sig. diff. ($P = 0·05$) .		0·10			0·01	
Stem						
1·0 daylight . . .	0·28	0·29	0·34	0·19	0·21	0·24
0·5 ,, . . .	0·29	0·33	0·37	0·22	0·26	0·29
0·24 ,, . . .	0·34	0·38	0·43	0·25	0·27	0·32
Sig. diff. ($P = 0·05$) .		0·01			0·01	
Leaves						
1·0 daylight . . .	0·43	0·44	0·44	0·46	0·48	0·49
0·5 ,, . . .	0·42	0·43	0·43	0·45	0·46	0·47
0·24 ,, . . .	0·42	0·14	0·40	0·45	0·46	0·48
Sig. diff. ($P = 0·05$) .		0·01			0·01	

The mean proportions of root, stem, and leaf after transference are given in Table I for expts. 32 and 33, and inspection shows that plants subjected to full daylight in the initial period tend to have the largest proportion of roots. Therefore, if a higher root ratio is associated with a greater respiration, it follows that since the net assimilation rates of plants originally not shaded are the same as those of shaded plants possessing less roots, then the actual assimilation of the 'sun' leaves will be higher. There is, however, another consideration which must be taken into account—that is how far the respiration of the roots or of the whole plant is linked with the level of respiratory substrates. Once more there does not appear to be direct evidence as to how far the reduced carbohydrate content and other associated changes in the composition brought about by shading affect the respiration in different tissues. It is improbable that these metabolic changes would increase the respiration; it is more likely that they would depress it. On this basis it can be advanced that the plants which initially received full daylight combined a larger proportion of actively respiring root with an overall higher level of respiration, and therefore a higher rate of carbon fixation in the leaves. It is clear that this evidence relating assimilation with the leaf area to weight ratio is somewhat tenuous and further investigation on this point will be required. At present, it is questionable whether the correlation is high. Both between experiments and between the light intensities after transference the residual effects of the initial light treatments on leaf modification and on the proportionate changes in root, stem, and leaf weight can show considerable variation without any change in the net assimilation rate.

It can been shown that shading leads to differential changes in the growth rates of the root, stem, and leaf, and the question arises of how the light factor operates in bringing about this reorientation of the relative growth rates of the different parts. It is generally held that the organization of growth is dependent on both the distribution of plant growth substances and the partition of the essential substrates for cell formation and differentiation. At the present time, much interest is being taken in the physiological role of growth regulators and the various aspects have been the subject of a recent symposium (Skoog, 1951) and reviews, for example, by Parker and Borthwick (1950), Larsen (1951), and Bonner and Bandurski (1952). It is not therefore proposed in discussing the interdependence of light and the growth substances to refer in detail to all the numerous papers.

Although indolylacetic acid has been identified in plant tissues and although it has been shown experimentally that this compound can control growth and development, the dependence of some of the changes on this substance alone is now debatable. Went (1951) has discussed the case for a leaf-growth regulator while Bouillenne (1950) has postulated that the development of roots can only be satisfactorily explained on the basis of a specific complex. More recently, the presence of 3-indolylaceto-nitrile in brussels sprouts and other crucifers has been demonstrated by Jones, Henbest, Smith, and Bentley (1952). By chromotographic techniques Bennet-Clark, Tambiah, and Kefford (1952) have shown that in etiolated sunflower seedlings besides indolylacetic acid there is also a growth inhibitor, while Luckwill (1952) examining different plant tissues records the presence of a varying number of compounds with growth-promoting or growth-inhibiting activities. Thus the development of a particular organ is likely to be dependent on the overall interaction of several compounds; in consequence any effect observed as a result of the addition of any one growth regulator is a net effect of its interaction with other auxins or inhibitors.

Although for many species it would seem that the auxin content of roots is optimal, there is evidence that application of indolylacetic acid to intact roots may in some species lead to increased growth. Naundorf (1940) records that as a result of the illumination of the roots of *H. annuus* their growth and auxin content was augmented. On the other hand, roots are very sensitive to indolylacetic acid and the amounts which may not affect stem growth inhibit root growth. This differential effect of indolylacetic acid on young sunflowers grown in the open has been demonstrated during the course of another investigation which has been concerned with the effects of light and temperature on the activity of synthetic growth regulators and of which some aspects have already been briefly reported (Robertson-Cuninghame and Blackman, 1952). A varying number of droplets of a 0·3 per cent. solution

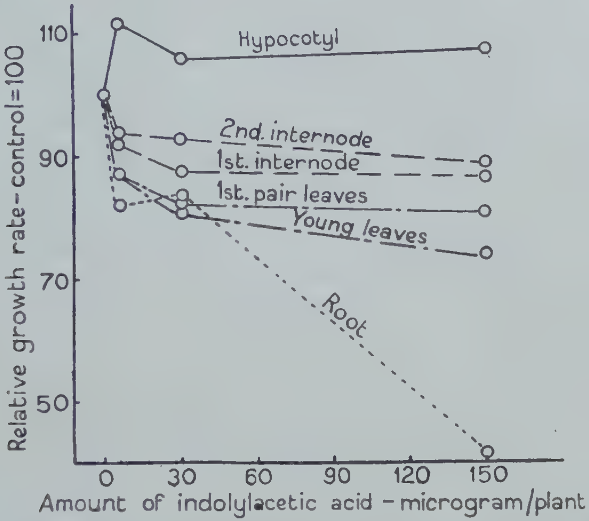

FIG. 13. The effects of indolylacetic acid, applied to the first pair of leaves, on the growth of the stem, leaves, and roots of *Helianthus annuus*. Growth rates expressed as percentages of the controls.

(equivalent to 3, 30, and 150 microgrammes) were added daily to the first pair of leaves and the plants harvested at the end of 8 days. From Fig. 13 it is evident that at the highest dose the percentage decrease in the relative growth rate was greatest for the root. Over all concentrations the growth of the young leaves and of the first and second internodes was significantly depressed but the apparent stimulus in the hypocotyl was not significant. In addition the length of both the first internode (immediately below the treated leaf) and the hypocotyl was reduced but the length of the second internode was not changed.

On the basis of these results it could be put forward that one of the primary effects of shading is to shift the content of growth substances in the roots to a supra-optimal concentration which inhibits growth. In order, however, to explain the residual influence of the pre-transference intensities on root growth further postulates are required. For example, since the relative growth rate of roots after transference from 0·24 daylight to daylight is faster than the rate for plants maintained in daylight over both periods, it must be argued that in full daylight the supply of growth substances is not in fact optimal and that the increased root growth of the previously shaded plants reflects a carry over of either supplies or a higher rate of production of growth substances or precursors. Conversely, it should follow that plants originally exposed to full daylight contain after transference to 0·24 daylight less available growth substances in the roots than plants kept continuously in 0·24 daylight. Therefore, their rate of root growth should be higher because the mean concentration subsequent to shading would reach a less inhibiting level. In fact, this is not so—the root growth of the initially unshaded plants is less and even negative in two out of the three experiments.

Considering next the effects of shading on stem growth, it has been demonstrated that in all three experiments a reduction to 0·5 daylight has not depressed the relative growth rate while a further decrease to 0·24 daylight has not changed the growth rate in two out of three experiments—Fig. 8. Although the growth rates may be little affected, the relative growth and the length of the internodes have been increased as the light intensity falls. These trends are consistent with the generally accepted concept that the changes in the stems induced by shading must be associated with an increased supply of growth substances. Since the residual effects of light intensity in the pre-transference period are small and in general not significant, it must be assumed that the availability of auxins is largely determined by the light intensity received in the post-transference period.

A comparison of Fig. 10 with Fig. 12 shows that variations in the light level differentially affect leaf weight and leaf area. Reducing the intensity depresses the growth rate in terms of weight but not in terms of area, and as a result the ratio of leaf area to weight increases logarithmically with shading (Fig. 11). While on a weight basis the residual influence of the light level in the pre-transference period does not lead to large and consistent variations in the subsequent growth rate, yet for area the maximal rates after transference —particularly in expt. 33—are found in those plants which initially received full daylight. Thus the same factors cannot be operating in governing the changes in weight and in area.

Went (1951) in his review of the role of growth substances in leaf development concluded that the group of factors which control the growth of the mesophyll are different from those concerned with the growth of the veins and petiole. It is possible by applying indolylacetic acid to leaves to increase vein development without affecting the lateral expansion of the mesophyll and it would appear that common factors operate in bringing about stem elongation and vein development. Went found that in the sunflower the leaves of plants exposed to full sun are of a different shape and size to those of plants grown under reduced light intensities. At a level below 'light' shade there is a decrease in leaf size, the leaf form changes from cordate to elliptical, and the angles between the midrib and the lateral veins become more acute because the expansion of the mesophyll is depressed more than vein growth by such a level of shading. In Went's view light is needed to activate the complex of factors responsible for mesophyll development ('phyllocaline') and as the light intensity rises above a threshold value so the activity increases: on the other hand, the complex determining stem and vein growth ('caulocaline') is relatively independent of light.

In the present experiments a reduction in intensity to 0·5 daylight has tended to increase rather than to reduce the growth rate in leaf area (Fig. 12), and this trend for the leaf area to be larger at 0·5 daylight has also been observed in 10 out of 16 other shading experiments on this species. Indeed, between these experiments there is no consistent reduction in area until a level of 0·12 daylight is reached. In contrast, in the present and in all the other experiments the ratio of leaf area to leaf weight increases sharply and logarithmically as the intensity falls below full daylight. If, therefore, as Went postulates, the lateral expansion of the mesophyll between the veins is dependent upon a complex of growth factors whose activity is dependent on the light intensity, then the factors responsible for the change from a shade to a sun leaf structure, as reflected in the changes in the ratio of area to weight, are far more sensitive to the light factor. In fact the conclusion can be reached that the two sets of factors are relatively independent. Some support for this view is forthcoming from unpublished investigations concerned with the analysis of the effects of light intensity on the activity of 2:4-dichlorophenoxyacetic acid. In one experiment sunflower plants treated with a non-lethal dose were subsequently grown at three levels of light intensity (1·0, 0·5, and 0·25 daylight). At the end of 6 days at each of the intensities the growth regulator had depressed the leaf area by 22–24 per cent., yet the changes in the area to weight ratio induced by shading were very similar for both the treated and control series. That is, leaf expansion was diminished without affecting the sensitivity of the interaction between light and the balance between area and weight.

The nature of phyllocaline is still a matter of speculation and it may well be that the changes can be ascribed to the interaction of indolylacetic acid and essential substrates, such as adenine, without involving other unspecified auxins. On the available but somewhat scanty evidence the net auxin content of normal leaves varies both with the stage of development and the species, and it seems that a distinction must be made between diffusible and bound auxin. Again little information is forthcoming as to the effects of high intensities (equivalent to 0·25–1·0 daylight), but some workers have found that the content and production is increased by longer photoperiods.

Whether auxins or precursors are exported from the leaves, it seems clear that the transport is associated with the movement of organic nutrients and is minimal at night. Weintraub and Brown (1950), employing bean plants which had previously been placed in darkness for at least 24 hours, observed that when indolylacetic acid and a variety of synthetic growth regulators were applied to a leaf, translocation out of the leaf did not take place unless sugars were added at the same time. Similarly, Rohrbaugh and Rice (1949) and Davis and Smith (1950) working with 2:4-dichlorophenoxyacetic acid found that export from the leaves of previously darkened plants only took place when sugars were added or the leaves were exposed to strong light. In this laboratory preliminary experiments indicate that if droplets of an aqueous solution of sodium 2:4-dichloro-5-iodo-phenoxyacetate, labelled with radioactive iodine (I^{131}) are placed on a sunflower leaf, then at the end of 24 and 48 hours a smaller proportion of the absorbed material is exported from plants which have been shaded (0·12 daylight as against 1·0 daylight).

Since the results quoted in the previous paragraph suggest that shading restricts the rate of movement out of the leaves, then the supply of auxins or precursors to the stem should be restricted by shading, but there is also the question of whether a reduction in the light intensity decreases the rate of inactivation.

The mechanisms for the photo-inactivation of indolylacetic acid have been discussed in some detail by Borthwick and Parker (1950) and Larsen (1951). Galston and his co-workers (e.g. Galston, Bonner, and Baker, 1950; Galston and Baker, 1951) working principally with pea tissues have postulated that two enzyme systems are involved. Firstly, a flavoprotein which in the presence of oxygen and light is converted from a reduced to an oxidized form with the liberation of hydrogen peroxide and, secondly, a perioxidase which specifically oxidizes indolylacetic acid. The mechanism of the final oxidation is a matter of some controversy, but it seems clear that in these *in vitro* experiments the inactivation system is sensitized by blue light.

There is always the inevitable query of how far conclusions based on *in vitro* experiments are applicable to whole plants, and current work in this laboratory indicates that in relation to the utilization of indolylacetic acid by intact tissues the results for brei or allied preparations may be different. Certainly, the observed effects of light on whole pea stems made by other workers are somewhat divergent since in contrast to the *in vitro* findings elongation is arrested more by red than blue light. In addition there are data for other plants which indicate that the relative activity of different wave-lengths is dependent on the stage of development. Moreover, although a change from darkness to weak light retards elongation, it has been repeatedly observed and has been found in the present experiments that at high light intensities shading leads to a greater relative stem growth and longer internodes. If the assumption is made that indolylacetic acid is the principal growth regulator involved, then the progressive elongation of the internodes could be accounted for on the further postulates that (i) the auxin content is optimal in darkness and supra optimal and inhibiting in full daylight, and (ii) that between these two limits the content is dependent on the rate of inactivation which is directly linked with the level of light intensity. The data for Fig. 13 lend some support to the contention that in full daylight the content is supra-optimal since the lowest dosage and even lower dosages in other experiments tend to reduce the length of the first internode and hypocotyl and to decrease the growth rates of the internodes. On the basis of these postulates it should follow that at low light intensities the addition of indolylacetic acid or other growth regulators should inhibit the growth or elongation of stem tissue, but this is contrary to much experimental evidence where an increased elongation has been obtained. That is to say, the auxin content is suboptimal and not supra-optimal.

If the auxin level is to be maximal in darkness and also in full daylight, then it could be advanced that there is another light-controlled mechanism which either inhibits the mechanism of photo-inactivation or controls the rate of production of indolylacetic acid from some precursor. On such assumptions transference from darkness would so alter the equilibrium that the supply of the auxin changes from a near optimal to a suboptimal level while at high intensities shading would bring about a shift from a supra-optimal and growth inhibiting concentration to a lower growth accelerating content. Such a shift need not necessarily be solely controlled by metabolic reactions in the stem tissues but also by a reduced supply of auxin or precursor coming from the developing leaves and apex.

It must, however, be emphasized that these postulates like those of previous workers are based on the assumption that only one growth regulator is involved, and in view of the findings of Bennet-Clark *et al.* (1952) that there is a growth-inhibiting compound in sunflower tissue it would seem that the difficulties of interpretation may well arise from considering a system in which there is a single growth regulator. In this connexion, investigations not yet published on the interaction between indolylacetic acid and dichlorophenoxyacetic acid have shown that at high light intensities mixtures of the two compounds may increase the growth of some of the internodes of sunflowers and the results are incompatible with the explanation that the sole function of the dichlorophenoxyacetic acid is to alter the availability of indolylacetic acid.

From the foregoing discussion it is apparent that much more research is required before the role of the light factor in controlling vegetative development in the root, shoot, or leaves can be interpreted in terms of auxin supply. Apart, however, from the problems of the regulation of growth by auxins there is also the question of light intensity and the differential mobilization of substrates essential for cell production.

It has been established that when plants initially grown in full daylight are transferred to 0·24 daylight then the relative growth rate of the roots may be negative (Fig. 6, expts. 31 and 32). Such a loss in weight could arise in two ways: either the quantities of substrates reaching the root system did not balance the respiratory losses, or there was an actual transference of materials out of the root into the shoot. The results of an earlier investigation would suggest that export from the root is likely to have taken place. Blackman and Templeman (1940) found that if established plants of *Agrostis tenuis* immediately after close defoliation are grown under three levels of light (1·0, 0·61, and 0·44 daylight), then at the end of 10 days the total sugar content and weight of the leaves *rises* as the light intensity falls. However, when at this point the plants are again defoliated, then 10 days later the new regenerated leaves weigh less and contain less sugar at the lower intensities. Such a reversal in trend can best be accounted for in terms of the mobilization of reserves in the stem bases and roots and their transference after the first defoliation to the regenerating leaves.

In the present experiments it has been found that after transference from one light intensity to another then a new equilibrium is reached within a relatively short time. On the basis of the trends for leaf-area ratio adjustment is complete at the end of 8 days (Fig. 1). Such a result differs markedly from the much less rapid rate of readjustment recorded for *Scilla non-scripta* (Blackman and Rutter, 1948). Since the bluebell grows more slowly and all the leaf primordia are laid down in the autumn prior to their emergence in the spring, a lower degree of plasticity is to be expected. It is, however, of interest that although the shading screens were not put in place until after emergence the leaf area to weight ratio could still be altered by the light treatment. An analogous result, which will be described in more detail in a later paper, has been obtained with *Fagopyrum esculentum*. When very young seedlings with only developing cotyledons (mean area per cotyledon 1·25 cm.^{2}) are shaded, then the area : weight ratio of the expanding cotyledons is still inversely proportional to the logarithm of the light intensity.

Ashby and Wangermann (1950) in their morphogenetic studies of the leaves of *Ipomaea caerulea* record that cell formation, based on counts of epidermal cells, is complete before the individual leaves reach 2·0–3·3 cm.^{2} If there is a similar trend in the sunflower, then most of the changes in the leaves observed in these experiments will have taken place during the phase of cell expansion. In this respect, therefore, the conditions under which shading increases the area : weight ratio are similar for the sunflower, the bluebell, and the cotyledons of *F. esculentum*. It is only possible at present to speculate as to how far the changes after cell formation are controlled by growth substances or the level of substrates which in turn will be dependent on the light intensity and the competitive demands of the other organs. Went (1951) has obtained evidence that in peas the growth factors responsible for leaf development are stored in the cotyledons and are exported to the developing leaves. If this is so for *F. esculentum*, then it would seem that in the regulation of cotyledon development a reduction in the available substrates is more important than a variation in the supply of growth substances.

With falling light intensity the level of substrates in the leaves is made up of two components: the reduction due to a lower level of carbon fixation and the withdrawal from the leaves of material for the growth of the stem or root. What is not yet known is whether there is a *pro rata* distribution or redistribution of substrates in proportion to the development of the different organs, or whether for the tissues which start into active growth there is localized accumulation of metabolites in excess of immediate utilization. The fact that within a short time of transference from daylight to 0·24 daylight the root of the sunflower may lose weight or that the regenerating leaves of *A. tenuis* under shade contain more sugar than those exposed to full daylight suggests that localized mobilization is one of the factors which must be taken into account in the further elucidation of the interrelationship between light intensity and plant development.

Lastly, there remains for discussion the significance of the present findings in the interpretation of changes in the environment. The horticultural implications have been reviewed in a recent paper (Blackman, 1953). It has already been pointed out (pp. 71–72) that in nature when there is a relatively abrupt change from one light level to another it is from a lower to a higher intensity. Under these conditions there should be an accelerated rate of growth, as long as other factors such as nutrient and water supply are not limiting. The period of a higher growth rate will be dependent on the species and the stage of development at the time of transference. The period will be least when the growth rate is high and the alteration in intensity takes place before a large proportion of the leaves has been formed—that is to say, when the potential of plastic adaptation is maximal. Conversely, the higher growth rate will persist longest for species which exhibit little plasticity. Thus in a mixed community the sudden removal of shade conditions may for the time being alter the balance in favour of the less plastic species.

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Stamen Number and Variation in Diploid and Tetraploid Cherries

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ABSTRACT

Stamen numbers were counted on flowers from a collection of cherries, sweet cultivated varieties of *Prunus avium* and *P. cerasus*. The most frequent numbers were 34 and 39–40, no doubt representing seven and eight times the petal number. The character is apparently genetically controlled, clones being highly uniform within themselves but differing from others.

On the average, flowers of tetraploid Morellos (*P. cerasus*) had lower numbers than diploid sweet cherries (*P. avium*), while tetraploid Dukes were intermediate; this accords with the suspected hybrid origin of the Dukes. Stamen number and variation was not associated with economic characters, but there was a tendency for varieties in more frequent incompatibility groups I–VI to have lower values than groups VII–XII.

INTRODUCTION

CULTIVATED cherries have five petals per flower, although six petals occasionally occur. Initial examination of stamen number in cherry flowers indicated that they were neither in multiples of petal number nor infinite, but had definite range patterns. The Rosaceae, according to Willis (1919), characteristically have stamens either two, three, or four times as many as the petals, usually five, or an infinite number. A detailed examination was therefore made of stamen frequency in cultivated diploid ($2n = 16$) sweet cherries (*Prunus avium*), tetraploid ($2n = 32$) Duke, and Morello cherries (*P. cerasus*).

In his account of varietal recognition Grubb (1949) makes no reference to this character. He states that whereas fruit characters are important in apples, pears, and plums, there are whole groups of cherry varieties with fruits so similar that they cannot be readily distinguished; hence various tree characteristics are used. It was therefore decided to test whether number and variation in this quantitative character can be used as an additional diagnostic character in identifying cherry varieties. Also it would be interesting to see if diploid and tetraploid cherries are separable by stamen number and whether this sheds light on the origin of the Dukes. Furthermore, it would be helpful to know whether stamen number among cultivated sweet cherries is associated with incompatibility groups.

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SAMPLING AND MATERIAL

As stamen counting is somewhat laborious, it was necessary to know the minimum number of flowers that may safely be used to give a reliable average, and which also would clearly show the variation within the variety. Table I shows the effect of sample size on average stamen number and variation within a tree of 'Merton Bounty'. Standard deviations ($S.D.$) were calculated in the usual way, and coefficients of variation (C) from the formula,

$$\left(C = \frac{100 \times S.D.}{\text{Mean}}\right)$$

It was decided that ten flowers per tree provided a satisfactory statistic for each variety. The ten flowers were collected at random from various inflorescences on various branches; old flowers occasionally showed signs of bird damage. In some varieties the innermost whorl of small stamens occur within the thalamus, and care was taken to include them.

TABLE I

Effect of Sample Size on Mean and Variation of stamens in One Cherry Variety

Number of flowers.	Mean stamen number.	Standard deviation.	Coefficient of variation.
5	31·0	1·9	6·1
	31·2	0·8	2·6
	31·4	1·1	3·5
	31·4	2·4	7·6
	31·6	1·5	4·7
	32·0	1·6	5·0
10	31·1	1·4	4·5
	31·5	1·9	6·0
	31·7	1·3	4·1
15	31·2	1·3	4·2
	31·6	1·8	5·7
20	31·4	1·4	4·5
25	31·4	1·4	4·5
30	31·4	1·5	4·8

The trees used were mostly pot plants of the Institution's collection of cherry varieties kept for testing incompatibility groups; they are without doubt correctly named. Most varieties are on F 12/1 Mazzard stocks, some on F 2/1, and a few on F 5/5. A small collection of trees of popular varieties grown in the field was also examined. Mean stamen number and variation for each diploid and tetraploid variety are classified in Table II according to cultural conditions and year when the counts were made. When more than one tree of a clone had been counted, the lowest value for each only was included when calculating sample means and standard deviations.

TABLE II

Stamen Numbers and Variations in Cultivated Cherries

Means are based on 10 flowers per tree; Greek letters indicate different trees in a clone

I. DIPLOID SWEET CHERRIES

	1951			1952		
Variety.	Mean stamen number.	Standard deviation.	Coefficient of variation.	Mean stamen number.	Standard deviation.	Coefficient of variation.
1. Pots in glasshouse						
Napoleon . .	30·0	0·4	1·3	—	—	—
Werder's Early Black	30·3	1·4	4·6	30·3	0·7	2·3
White Heart . .	30·4	0·8	2·6	34·4	2·3	6·7
Black Tartarian A .	30·7	1·4	4·6	—	—	—
Merton Bounty α .	31·0	1·6	5·2	—	—	—
,, ,, β .	31·1	1·4	4·5	—	—	—
Frogmore Early .	32·2	1·6	5·0	—	—	—
Early Rivers . .	32·3	2·5	7·7	33·3	2·8	8·4
Black Circassian .	32·6	3·4	10·4	36·9	2·6	7·0
Emperor Francis .	32·7	2·3	7·0	40·0	1·1	2·8
Caroon B . .	32·9	2·1	6·4	—	—	—
Merton Bigarreau .	33·3	1·3	3·9	—	—	—
Bedford Prolific A α .	33·4	1·7	5·1	—	—	—
Van . . .	33·8	2·0	5·9	31·0	1·2	3·9
Bedford Prolific A β .	34·7	2·0	5·8	35·4	2·8	7·9
Caroon A . .	35·3	1·9	5·4	38·8	2·3	5·9
Roundel . . .	35·6	2·0	5·6	—	—	—
Peggy Rivers . .	35·8	2·3	6·4	—	—	—
Kentish Bigarreau .	36·1	3·0	8·3	—	—	—
Guigne de Winkler* .	36·1	1·6	4·4	—	—	—
Sparkle . . .	36·2	1·8	5·0	—	—	—
Nutberry Black .	37·1	2·0	5·4	34·6	1·5	4·3
Bigarreau Gaucher .	37·2	2·1	5·6	—	—	—
Merton Premier .	37·4	2·8	7·5	—	—	—
Merton Heart α .	37·7	2·6	6·9	—	—	—
,, ,, β .	38·1	2·1	5·5	—	—	—
Wellington A . .	38·1	2·2	5·8	—	—	—
Late Black Bigarreau.	39·5	1·4	3·5	39·6	2·0	5·1
Merton Glory . .	39·9	1·2	3·0	39·0	1·1	2·8
Rodmersham . .	40·4	2·5	6·2	—	—	—
Bigarreau de Schrecken	40·9	1·4	3·4	—	—	—
Governor Wood .	41·3	2·9	7·0	—	—	—
Ramon Oliva . .	—	—	—	31·5	2·0	8·9
Cleveland Bigarreau .	—	—	—	32·5	2·0	6·1
Bowyer Heart . .	—	—	—	37·3	1·2	3·2
MEAN . .	35·1±3·3			35·7±3·0		
2. Pots in Frameyard						
'1964' . . .	27·9	2·7	9·7 †	—	—	—
Mezel No. 2 . .	29·1	1·4	4·8	—	—	—
Maiden's Blush .	29·6	2·0	6·8	—	—	—
Mumford Black .	30·0	2·9	9·7	—	—	—

* Synonym: Belle Agathe.

† Flowers of '1961' and '1964' were abnormal.

TABLE II (*cont.*)

	1951			1952		
Variety.	Mean stamen number.	Standard. deviation.	Coefficient of variation.	Mean stamen number.	Standard deviation.	Coefficient of variation.
Frogmore Bigarreau .	31·4	1·6	5·1	30·4	1·2	3·9
Napoleon α . .	31·6	1·7	5·4	—	—	—
'1961' . . .	31·7	4·2	13·2†	—	—	—
Merton Bigarreau α .	32·6	2·5	7·7	—	—	—
Ludwig's Bigarreau .	32·6	2·1	6·4	30·8	2·5	8·1
Merton Bigarreau β .	32·8	1·4	4·3	—	—	—
Napoleon β . .	33·2	2·4	7·2	—	—	—
Peggy Rivers . .	33·4	1·6	4·8	—	—	—
Early Rivers . .	33·6	2·5	7·4	—	—	—
Late Amber . .	33·7	1·8	5·3	29·8	1·8	6·0
Van . . .	34·0	2·6	7·6	—	—	—
White Bigarreau .	34·2	1·0	2·9	—	—	—
Newington Black .	34·6	1·7	4·9	—	—	—
Late Black Bigarreau.	34·6	2·7	7·8	—	—	—
Turkish Black . .	34·9	2·2	6·3	—	—	—
Knight's Early . .	35·7	2·5	7·0	—	—	—
Ord American . .	35·7	2·2	6·2	—	—	—
Governor Wood .	36·0	2·1	5·8	—	—	—
Weston's Amber .	36·2	4·2	11·6	—	—	—
Merton Heart . .	36·6	3·0	8·2	39·9	1·3	3·3
Bigarreau Gaucher .	37·0	1·4	3·8	—	—	—
Florence Rivers .	37·1	1·9	5·1	—	—	—
Hooker's Black. .	37·3	2·5	6·7	—	—	—
Nutberry Black .	37·4	1·8	4·8	—	—	—
Guigne de Winkler* .	37·5	2·4	6·4	33·5	2·4	7·2
Leicester . . .	37·9	2·3	6·1	35·0	3·1	8·9
Bradbourne Black .	38·2	2·1	5·5	—	—	—
Emperor Francis, Rivers . . .	38·2	1·3	3·4	—	—	—
Judy's Fancy . .	38·4	2·1	5·5	37·3	2·0	5·4
Kassin's Frühe Hertz	38·4	1·9	4·9	—	—	—
Knight's Bigarreau .	39·2	3·6	9·2	33·8	2·0	5·9
Emperor Francis .	39·5	1·6	4·1	40·6	2·7	6·7
Cryall's Seedling .	39·8	1·2	3·0	40·4	2·4	5·9
Reidern 2nd Early Black . . .	40·4	3·2	7·9	—	—	—
Ursula Rivers . .	44·7	1·5	3·4	—	—	—
MEAN . .	35·3±3·5			34·7±4·3		
3. Trees in field						
Werder's Early Black	30·4	1·0	3·3	29·8	1·7	5·7
Caroon B . .	31·8	1·9	6·0	34·7	1·8	5·2
Merton Bounty .	33·1	2·8	8·5	31·0	2·5	8·1
Emperor Francis .	34·3	3·8	11·1	—	—	—
Merton Bigarreau .	34·3	3·2	9·3	—	—	—
Napoleon . .	34·4	2·3	6·7	—	—	—
Black Circassian .	36·2	1·5	4·1	—	—	—
Wellington A . .	36·7	2·6	7·1	33·1	2·1	6·3
Rodmersham Seedling	36·7	2·8	7·6	35·0	2·1	6·0
Nutberry Black .	38·7	1·4	3·6	—	—	—
Wellington B . .	—	—	—	30·9	1·7	5·5
MEAN . .	34·7±2·5			32·4±2·2		

* Synonym: Belle Agathe.

† Flowers of '1961' and '1964' were abnormal.

II. TETRAPLOID ACID AND SOUR CHERRIES

	1951			1952		
Variety.	Mean stamen number.	Standard deviation.	Coefficient of variation.	Mean stamen number.	Standard deviation.	Coefficient of variation.
	1. *Pot in glasshouse*					
Planchoury . .	36·1	2·7	7·5	—	—	—
	2. *Pots in frameyard*					
Morello A . .	26·8	1·7	6·3	27·9	1·8	6·5
Kentish Red Morello	30·1	2·8	9·3	—	—	—
Wye or Kentish Morello . .	30·2	0·8	2·6	27·8	3·1	11·2
Archduke . .	31·3	4·2	13·4	29·4	2·3	7·8
Reine Hortense .	31·6	1·3	4·1	—	—	—
Late Duke α . .	32·1	1·1	3·4	31·4	1·5	4·8
,, ,, β . .	32·7	2·3	7·0	—	—	—
Ostheimer Weichsel .	32·7	3·3	10·1	—	—	—
May Duke . .	34·2	1·3	3·8	30·8	3·2	10·4
Royal Duke . .	34·5	2·7	7·8	—	—	—
MEAN . .	31·5±2·3			29·5±1·6		

THE GENETICAL BASIS

A test was made to determine the variation within genetically uniform clones. Table III gives the data for two trees each of 'Merton Heart', 'Merton Bounty' and 'Bedford Prolific' A raised in pots in a glasshouse. The means for each variety are readily distinguishable, e.g. 'Merton Heart' averages six to seven stamens per flower more than 'Merton Bounty'. Trees within each clone hardly differ in their means, and their standard deviations are similar. There is thus only slight variation within genetically uniform clones that have been grown in the same environment.

TABLE III. *Stamen Variation within Clones*

		Number of flowers with																
Variety.	Tree no.	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	Mean.	*C.*
Merton Bounty	68	1	0	1	4	3	1	—	—	—	—	—	—	—	—	—	31·1±1·4	4·5
	67	—	2	2	3	1	1	1	—	—	—	—	—	—	—	—	31·0±1·6	5·2
Bedford Prolific A	α	—	—	—	2	1	2	2	2	1	—	—	—	—	—	—	33·4±1·7	5·1
	β*	—	—	—	1	1	0	1	2	3	1	—	—	—	—	—	34·7±2·0	5·8
Merton Heart	72	—	—	—	—	—	—	1	0	1	1	3	1	2	1	—	38·1±2·1	5·5
	73	—	—	—	—	—	1	0	1	1	2	0	3	1	0	1	37·7±2·6	6·9

* Stamens in 9 flowers only were counted.

To determine the yearly variation in a clone, counts were made in 1951 and 1952 on six trees of variety No. 1464, all grafted on to the same rootstock. These data are given in Table IV. Again there are no significant differences between members of a clone, but trees in 1952 had slightly lower means.

In the item for 'years', $t_{[5]} = 2{\cdot}36$ and, as P at 0·05 is 2·57, it is on the borderline of significance. Annual differences in stamen number must therefore be taken into account, especially when there are wide differences in annual temperature.

Table IV

Annual Stamen Variation in Six Trees of One Clone

Tree.	Year.	Number of flowers with												Mean.	*C.*
		25	26	27	28	29	30	31	32	33	34	35	36		
α	1951	—	—	—	—	—	1	1	1	2	3	1	1	33·2±1·8	5·4
	1952	—	—	—	1	0	1	3	2	1	1	1	—	31·7±2·0	6·3
β	1951	—	1	0	0	2	2	1	2	1	1	—	—	30·6±2·3	7·5
	1952	—	—	—	2	1	1	2	3	1	—	—	—	30·6±1·8	5·9
γ	1951	—	—	—	—	2	2	1	1	2	1	0	1	31·7±2·3	7·3
	1952	—	—	—	1	0	2	4	2	1	—	—	—	30·9±1·4	4·5
δ	1951	—	—	—	1	1	3	3	2	—	—	—	—	30·4±1·3	4·3
	1952	1	0	0	0	1	5	1	0	1	1	—	—	30·2±2·4	7·9
ε	1951	—	—	—	—	1	2	0	0	2	4	0	1	32·7±2·3	7·0
	1952	—	—	—	—	2	5	1	2	—	—	—	—	30·3±1·1	3·6
φ	1951	—	—	—	1	0	2	4	1	1	1	—	—	31·1±1·7	5·5
	1952	—	—	—	1	1	3	3	0	1	1	—	—	30·7±1·8	5·9

Analysis of Variance

Item.	S.S.	N.	M.S.	V.R.	*T.*	*P.*
Trees . . .	583·8	5	116·8	2·8	—	Not significant
Years . . .	234·1	1	234·1	—	2·36	Borderline significance
Trees-years . .	208·4	5	41·7	—	—	
Total . . .	1026·3	11				

Table V gives the segregation for stamen number in young trees derived from a tree of ('Bigarreau de Schrecken' × 'Governor Wood') selfed. The analysis of variance, calculated by the method in Haskell (1949), clearly shows that these progeny significantly differ in stamen number. These differences must be genetically controlled, as they were all raised in the same environment. 'Bigarreau de Schrecken' averaged 40·9 and 'Governor Wood' 41·3 stamens. The range of their offspring following hybridization and selfing varied from 32·8 to 42·9. The lower values could be due either to polygenic segregation or to inbreeding depression in a species adapted for cross-pollination. It is also clear that the pattern of variation is specific for each seedling, some having more internal variation than others.

ENVIRONMENTAL EFFECTS

Several analyses were made on factors most likely to effect stamen production to find out the influences of environmental and cultural conditions.

Annual variation

Variation in stamen frequency from year to year is given in Table VI. The figures are based on the averages of different varietal means grouped according to environment and ploidy. It is seen that 1952 values for trees grown outside were slightly less than those for 1951, usually by a little over one

TABLE V

Segregation for Stamen Number in Sibs from Tree of ('Bigarreau de Schrecken' × 'Governor Wood') selfed

	Number of flowers with																					
Seedling No.	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	Mean.	*C.*
2539 . . .	2	0	1	1	2	1	2	0	1	—	—	—	—	—	—	—	—	—	—	—	32·8±2·6	7·9
2545 . . .	1	0	0	0	3	3	2	0	1	—	—	—	—	—	—	—	—	—	—	—	33·7±2·1	6·2
2540 . . .	—	—	—	—	1	0	0	0	0	2	2	3	1	1	—	—	—	—	—	—	39·0±2·4	6·2
2538 . . .	—	—	—	—	—	—	—	—	—	—	2	2	3	2	1	—	—	—	—	—	40·8±1·3	3·2
2541 . . .	—	—	—	—	—	—	—	—	—	—	2	0	4	1	1	1	0	0	1	—	41·8±2·4	5·7
2537 . . .	—	—	—	—	—	—	—	—	—	—	1	0	1	4	1	0	2	0	0	1	42·9±2·5	5·8
Total . .	3	0	1	1	6	4	4	0	2	2	7	5	9	8	3	1	2	0	1	1	38·5±2·3	6·0
Bigarreau de Schrecken	—	—	—	—	—	—	—	—	—	—	1	4	2	2	0	1	—	—	—	—	40·9±1·4	3·4
Governor Wood .	—	—	—	—	—	—	—	—	1	1	1	2	0	1	1	1	2	—	—	—	41·3±2·9	7·0

Analysis of Variance

Item.	S.S.	N.	M.S.	V.R.	*P.*
Between seedlings . .	585·8	5	117·2	5·2	< 0·001
Within seedlings . .	1220·2	54	22·6		
Total . .	1806·0	59			

stamen. On the other hand, glasshouse trees had somewhat higher stamen numbers in 1952. On the whole, stamen number during 1951 and 1952 were similar. Unfortunately it was not possible to determine the effect of tree age on stamen production.

TABLE VI

Annual Variation in Cherry Stamen Frequency

Environment.	Ploidy.	No. of varieties.	Average stamen number		Difference.
			1951.	1952.	
Trees in field	$2n$	5	33·7	32·7	−1·0
Pot trees outside	$2n$	14	35·7	34·3	−1·4
	$4n$	5	30·9	29·5	−1·4
Pot trees indoors	$2n$	11	34·8	36·8	+2·0
		Mean	33·8	33·3	−0·5

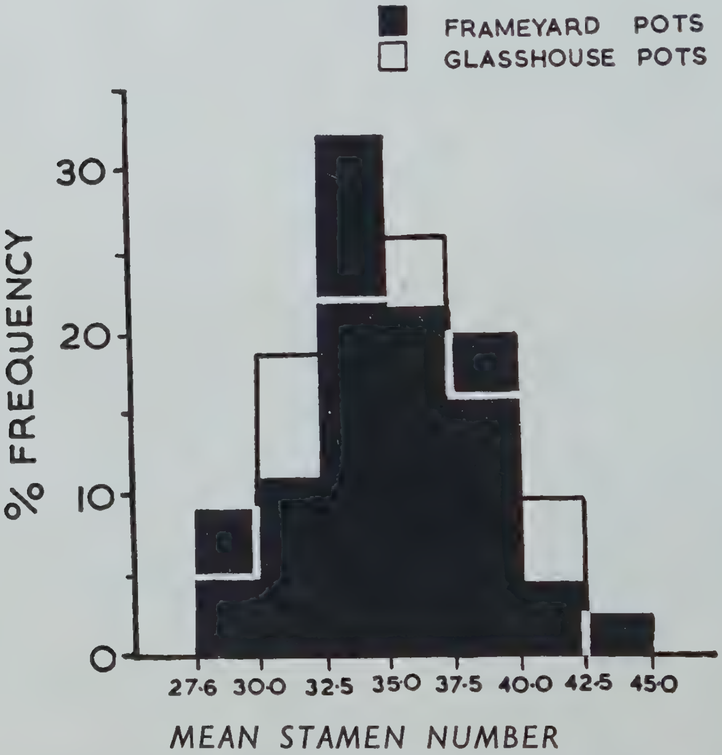

FIG. 1. Histograms showing frequency distributions of mean stamen number per sweet variety, in groups of 2·5 stamens, for pot plants in frameyard and glasshouse, 1951.

Pot trees grown inside and outdoors

Fig. 1 gives the histograms of frequency distributions of mean stamen number for pot plants of sweet cherries either grown in a glasshouse or outside. The samples consisted of 31 varieties inside and 46 outside, no variety being duplicated. It is seen that there is little over-all difference between the samples. The mode is 32·6 to 35·0 for outdoor plants and 35·1 to 37·5 for inside plants. The distribution for outside trees shows slight positive skewness, not seen in that for inside trees. Apparent lack of temperature influence during spring growth is probably due to flower-bud primordia having been laid down in the previous autumn (Roberts, 1917).

Pooled frequencies were plotted of actual stamen numbers for frameyard and glasshouse varieties. There were 31 sweet cherry varieties and one clone in glasshouse pots and 45 varieties in frameyard pots. A variety was included in both groups in a few instances. The distributions are those shown in Fig. 2. The mode is 34 stamens per flower for both environments, and there is a secondary peak at 39–40; these perhaps represent 7 and 8 times the numbers of petals. Thus the curves, which are similar, both show a tendency to bimodality.

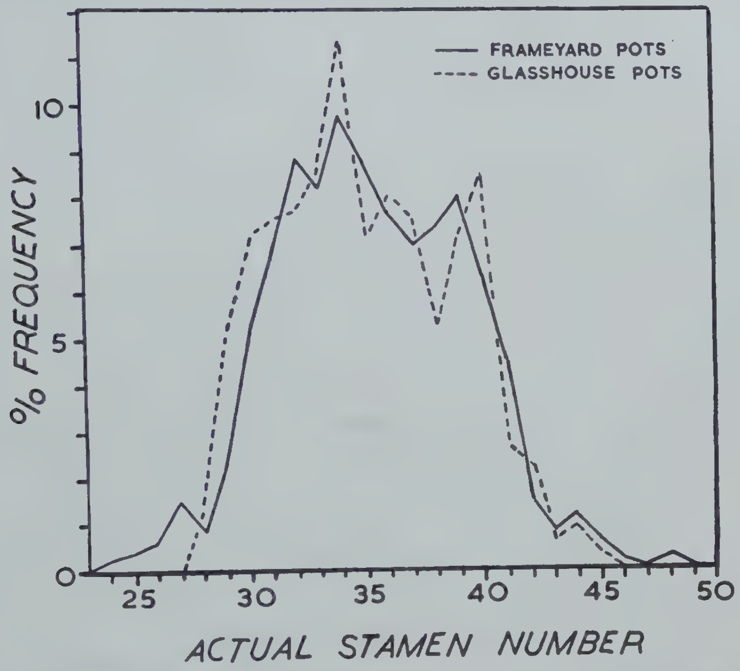

FIG. 2. Frequency distributions of actual stamen numbers for 31 sweet varieties in the glasshouse, and 45 sweet varieties outside, in pots.

Pot and field trees

Fig. 3 gives the correlation between 10 sweet cherry varieties in 1951, for trees grown in glasshouse pots and raised as trees in the field. Although there is fair correlation, it is clear that field trees of some varieties had slightly higher stamen numbers, and in several instances had higher standard deviations. On the average, field trees had 34·7 stamens and those in pots inside had 33·9.

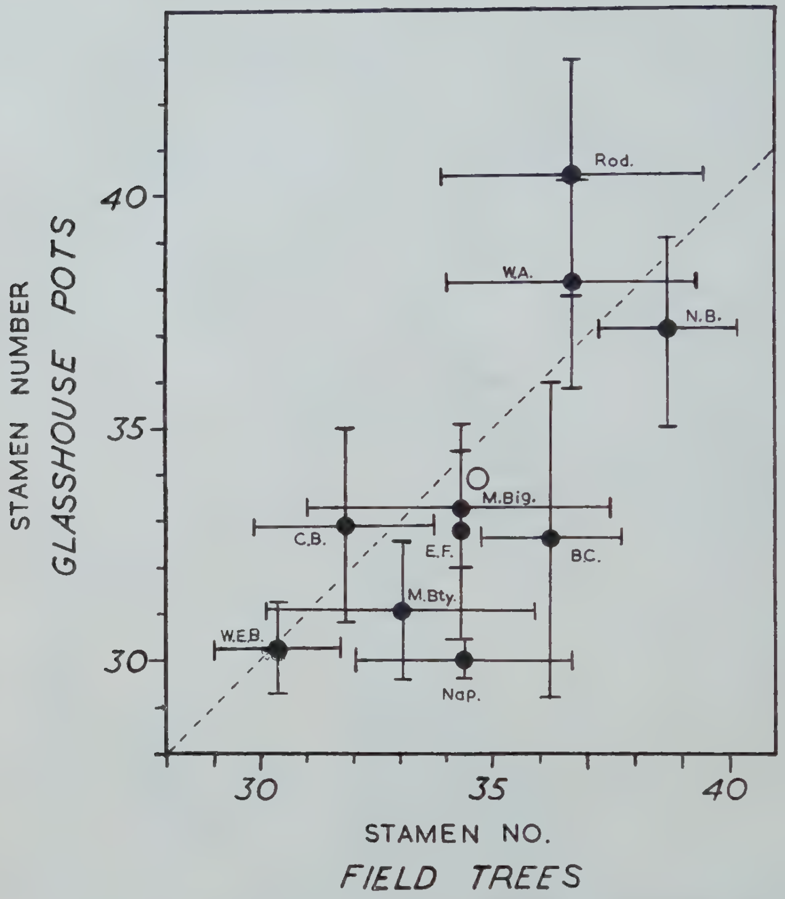

FIG. 3. Correlation between 10 sweet varieties for trees grown in glasshouse pots, 1951, and clones raised as trees in the field, 1951. Black spots = varietal means; open circle = average over all varieties; the distance between two bars along each line = 2 × standard deviation. The dotted line represents a theoretical correlation of +1. *Key to Varieties:* B.C., 'Black Circassian'; C.B., 'Caroon B'; E.F., 'Emperor Francis'; M.Big., 'Merton Bigarreau'; M.Bty., 'Merton Bounty'; Nap., 'Napoleon'; N.B., 'Nutberry Black'; Rod., 'Rodmersham Seedling'; W.A., 'Wellington A'; W.E.B., 'Werder's Early Black'.

Root-stocks

Root-stocks affect quantitative behaviour, such as flowering time. The two most commonly used mazzard root-stocks are Malling F 12/1 and 2/1. Only meagre data was available for testing: for pots in the frame in 1951, seven varieties on F 12/1 averaged $36{\cdot}0 \pm 3{\cdot}4$ stamens, while three different varieties on F 2/1 averaged $33{\cdot}2 \pm 3{\cdot}6$. It is unlikely that root-stocks greatly influence the wide variation in the means of cultivated cherries, as seen in Table II, unless they have had a detrimental effect on scion growth.

DIFFERENCES BETWEEN DIPLOIDS AND TETRAPLOIDS

The means and ranges in stamen numbers for diploids and tetraploids, classified according to tree environment, are given in Table VII. Even though the tetraploid is much smaller than the diploid sample, it is clear from Table VII that Morellos have considerably lower stamen number. On the other hand, Dukes roughly average the same as diploids, but their range in stamen number is considerably less, the maximum being 36·2 compared with 41·3 respectively. This may be due to the smaller sample size of the Dukes, but it may also have bearing on the history of tetraploids.

TABLE VII

Stamen Number Ranges in Diploid and Tetraploid Cherries

		Pots in glasshouse		Pots in frameyard	
Ploidy.	Year.	Mean.	Range.	Mean.	Range.
2*n* Sweets	1951	35·1	30·0–41·3	35·3	29·1–44·7
	1952	35·7	30·3–40·0	34·7	29·7–40·6
4*n* Dukes	1951	—	—	32·7	31·3–34·5
4*n* Morellos	1951	—	—	29·0	26·8–30·2

If coefficients of variation are plotted from Table II for varieties of diploids, Dukes and Morellos, based on pot plants in frames, 1951, it is found that tetraploids have equally wide range in variation as diploids. But whereas coefficients of variation for diploids cluster mainly between 4·8 to 7·0, those of tetraploids lie mainly between 2·7 to 4·1 and again 6·3 and 7·8. It is interesting that the variation of one tetraploid only lies within the range for most diploids; perhaps this situation would alter if more data were available.

NUMBER AND VARIATION IN RELATION TO OTHER CHARACTERS

A number of analyses were made to determine whether stamen number and variation were associated with any of the more interesting commercial characteristics of modern cherries.

Flowering period and fruit picking times

Averages of stamen number of indoor and outdoor pot plants were calculated for varieties classified according to their time of flowering. Early varieties on the average had 36·3, medium flowering varieties had 35·3, and late varieties had 34·5 stamens. Thus although there is slight indication of a reduction in stamen number with later flowering, the difference of less than two stamens has no practical value.

The mean stamen number for sweet cherries graded according to seven commercial seasons of fruit ripening showed no relationship between these two quantitative characters.

Fruit colour

Grubb (1949) classified the two main groups of sweet cherries according to whether the juice is coloured or not. There are, on the average, no stamen differences associated with this property. Forty varieties with coloured juice had 35·9 stamens, while 23 with uncoloured juice had 34·6.

Incompatibility group

Table VIII shows the incompatibility groups, *S* allele combinations when known, and average stamen number for various varieties. Unfortunately relatively small samples are included in each group. However, when means of groups I to VI are pooled, and also those of VII to XII, it is observed that the latter have somewhat higher stamen numbers, viz. 37·8 compared to 35·1. A *t* test gave $t_{[10]} = 2{\cdot}07$, so that *P* lies just above the 0·05 level. A test for the variances gave *V.R.* = 2·82 for n_1 and $n_2 = 5$, the 0·20 level being 2·2. Groups I to VI include a greater proportion of the commoner cherries (Crane and Brown, 1949).

TABLE VIII

Stamen Numbers and Incompatibility Groups

Incompatibility group		Average stamen number 1951		
Number.	*S* alleles.	Pots inside.	Pots outside.	Mean.
I	$S_1\ S_2$	33·2 (6)	35·7 (3)	34·5
II	$S_1\ S_3$	34·6 (5)	32·8 (5)	33·7
III	$S_3\ S_5$	31·4 (2)	35·2 (11)	33·3
IV	$S_2\ S_3$	36·8 (2)	35·0 (5)	35·9
V	$S_3\ S_4$	39·5 (1)	34·6 (1)	37·1
VI	$S_1\ S_4$	36·4 (3)	35·8 (3)	36·1
VII	?	—	37·8 (2)	37·8
VIII	$S_5\ S_7$	35·5 (1)	33·4 (1)	34·5
IX	?	34·2 (1)	44·7 (1)	39·5
X	?	40·4 (1)	—	40·4
XI	?	—	39·5 (2)	39·5
XII	?	35·3 (1)	34·6 (1)	35·0

Mean of Groups I–VI = 35·1±1·5
" " " VII–XII = 37·8±2·5

Figures in brackets refer to number of varieties on which average is based.

Bacterial canker

This disease often limits cherry growing. No association was detectable between stamen number and resistance to bacterial canker from a study of the data.

Fruit setting

Table IX was prepared to determine whether variation in stamen number of flowers within a variety is associated with its ability to set fruit following compatible pollinations, i.e. yielding capacity. The values for mean percentage fruit setting were calculated as averages of values for settings in compatible crosses, given by Crane and Brown (1937). It is clear that although different varieties have different capacities for setting fruit, and also widely vary in stamen variation within varieties, there is no relation between these two properties.

TABLE IX

Stamen Variation and Fruit setting

Variety.	*C.*	Mean % fruit set.	No. of compatible crosses used.
Napoleon	1·3	30·9	53
Bigarreau de Schrecken	3·4	21·0	44
Late Black Bigarreau	3·5	29·7	28
Belle Agathe	4·4	27·5	28
Black Tartarian A	4·6	11·6	14
Frogmore	5·0	32·0	41
Bedford Prolific	5·1	37·5	40
Bigarreau Gaucher	5·6	31·7	3
Ursula Rivers	6·4	19·1	17
Peggy Rivers	6·4	33·1	15
Governor Wood	7·0	38·4	39
Early Rivers	7·7	30·4	36
Kentish Bigarreau	8·3	17·9	30

DISCUSSION

The most frequent stamen number was 34, followed by 39 or 40. It is clear that stamen number is not a precise multiple of petal number, nor is it really infinite like Willis (1919) has indicated. Each variety, as Table II shows, has its own mean and variation pattern. These both must be genetically determined, for when trees of the same clone are grown in the same environment their flowers have similar means and variation, but differ from those of other clones (Table III). In addition, offspring segregate, as in Table V, to produce new forms with their own intrinsic stamen pattern. The segregations are continuously distributed and therefore are polygenically inherited. Single flowers are dominant to double in *Eschscholtzia californica* (Beatty, 1937); no correlation exists between petal and stamen numbers, although there is greater variation of both within doubles. In contrast, petal number of cherries is constant; no extra-petalous cultivated varieties were found. This is similar to the situation found in *Stellaria media* (Haskell, 1949). Polygenes controlling stamen number in various species from different families therefore seem to function independently of those controlling petal number, regardless of whether or not these are stable.

When revising North American wild roses, Erlanson (1934) used, among other criteria, stamen number as a diagnostic character in *Rosa*. The number in each species varied in some instances within a wide range, but was a reliable specific character. Polyploidy and stamen number were not related. Boulenger (1937) considered that stamen numbers in Old World species of *Rosa* were useful only in making natural groups of species, although sometimes they assisted in species determination. As Grubb (1949) has emphasized, it is difficult to make keys for cherry classification; hence stamen numbers could be used in several ways, e.g. as an additional diagnostic feature in varietal identification, and in aiding recognition of clonal material. They may also be helpful in nomenclature, especially with regard to synonyms which are frequent among cherries, and in separating two or more varieties grown under the same name.

Several examples may be given of applying stamen numbers to economic problems with cherry varieties. 'Frogmore Bigarreau' and 'Maiden's Blush' are similar with respect to juice, incompatibility group, flowering, and fruit picking times: they also have similar stamen numbers, standard deviations, and coefficients of variation. These data taken together strongly indicate that they are closely related. 'Mezel No. 2' and 'Napoleon' are in the same incompatibility group: in the present experiment, 'Mezel No. 2' had 29·1±1·4 stamens and 'Napoleon' had 31·6±1·7, so they are not significantly different in this respect. Likewise, it might be possible to clarify the situation for other co-incompatible varieties thought similar. As varieties in the same groups are likely to be related, it is not unexpected that they have similar stamen numbers. This character may also be helpful in assisting recognition of those trees rescued from Knight's Elton orchard, suspected as being 'Waterloo' or 'Roundel'. Indeed, owing to the confusion of varietal names in most cultivated fruits, as Brown (1952) has pointed out, stamen numbers may possibly assist in the nomenclature of plums, apples, and pears.

When describing a newly released cherry variety it may be advantageous to record its stamen number and variation. Thus in glasshouse pot plants of the new Merton cherries, described by Hart (1947), 'Merton Bounty' had 31·0±1·6, 'Merton Bigarreau' 33·3±1·3, 'Merton Premier' 37·4±2·8, 'Merton Heart' 37·7±2·6, and 'Merton Glory' 39·9±1·2. Thus stamen number can help to separate some of these new varieties, all of which are blacks except for 'Merton Glory'. These data also afford evidence of genetic segregation, for 'Merton Heart' is a seedling from ('Bigarreau Schrecken' × 'Elton Heart') and 'Merton Bounty' is from the reciprocal cross. Yet both are readily separable by stamen number when grown in the same environment.

Roberts (1917) found developmental differences in buds of sour cherries according to shoot position during September in Wisconsin. Flower formation is therefore initiated first in basal and terminal regions of stems; there are varietal differences. When taking the present samples, flowers were selected from various positions on each tree; only rarely were two flowers taken from the same inflorescence. Gardner (1935) found that in some bud sports of Montmorency cherries, flower-bud formation takes place over a comparatively long period, beginning one month after completion in normal trees. As stamen formation is laid down the previous late summer or autumn, this may modify results for glasshouse pot trees, for some trees had previously been grown in frames. Comparison of 1951 and 1952 data (Tables IV and VI) shows that temperature during the preceding summer influences stamen production. Mean maximum and minimum Greenwich temperatures were slightly lower during June to October 1951 than in 1950, and stamen production in 1952 was correspondingly slightly lower than in 1951. This suggests stamen number is directly related to temperature, though possibly rainfall also has an influence. It was not possible to study effects of tree age, but Roberts (1917) found arrested development in blossom buds on younger trees. This might act indirectly through temperature effects when buds are formed.

Hruby (1939) concluded from quadrivalent formation in Duke cherries that they behave cytologically as autotetraploids and their hybrids with *P. avium* like autotriploids. Hence it is not impossible that Dukes are autotetraploid forms of *P. avium*. Similarly, Raptopoulous (1941) concluded from chiasma frequency and quadrivalent formation that both Dukes and sour cherries are autotetraploids, the former of *P. avium* and the latter from a postulated species morphologically differentiated from *P. avium* but both having a common ancestor. As incompatibility reactions are not so strong in tetraploids, they are genetically likely to be more closely related than any two sweet cherry varieties. Table VII shows that Morellos are separate from Dukes and sweets on account of their low stamen numbers. Dukes, while being similar to sweets, have not such a wide range in number; especially they lack higher ones. These data, together with the intermediate morphology of the Dukes, can therefore be interpreted as supporting the alternative hypothesis of Darlington (1933), viz. that Dukes are derived from crosses between 4*x* Morellos and presumably unreduced gametes of 2*x* sweets. Grubb (1949) also believes that Dukes are hybrids between *P. cerasus* and *P. avium*. Acid and Duke cherries are separable from sweets both on tree and leaf characteristics, and their juice colour is either 'red' or 'black; but the distinction between acid cherries and Dukes is less certain.

Kostoff (1939) postulated that auto- and allo-polyploids often have greater internal variation than the diploids from which they came, at least shortly after becoming polyploids. In the present data more of the tetraploids than the diploids have high coefficients of variation, but actual ranges in variation are similar. Thus stamen variation in cherries offers no support of this hypothesis, unless it is assumed that internal variation in older polyploids has modified back to that of the original diploids. Then there is the possibility that variation, at least in polyploid cherries, indicates their age. It was also thought that stamen variation within flowers of a particular variety would indicate its ability to set fruit, as Mather (1950) with *Primula sinensis* and Haskell (1953) with sweet corn found in these outbreeding species greater internal variation associated with inbreeding depression. As stamen variation in a variety might therefore be an index of inbreeding, and hence yielding ability, it was tested against fruit setting. However, no relationship was established (Table IX).

It had also been hoped that stamen number and variation were correlated with other economic quantitative characters. Unfortunately this was not so, probably because one is here dealing with polygenic inheritance on all chromosomes and linkage is not easily obtainable. Thus when the sweets were grouped according to flowering time, differences were very small, although there was slight reduction with later flowering. Similarly, there was no apparent relation between stamen number with juice colour or time fruit are ripe. Yet there was indication that incompatibility groups I to VI tended to have lower stamen numbers than groups VII to XII. It may be possible to determine whether the *S* alleles actually influence stamen number, when more of them have been assigned to the incompatibility groups.

SUMMARY

1. Stamen numbers and variations were determined in diploid sweet and tetraploid Duke and Morello cherries. Stamen pattern is polygenically inherited: seedlings segregate but clones in the same environment are uniform. Each cultivated variety has its own intrinsic pattern.

2. Environmental studies show that: (i) annual variation is slight; (ii) there are only slight differences for pot trees indoors and outdoors; (iii) field trees have slightly higher stamen numbers than when indoors in pots; (iv) root-stocks are unlikely to have much influence.

3. Frequency distributions of actual stamen numbers in populations are bimodal, with a major peak at 34 stamens and a secondary at 39–40. Morellos have lower numbers, while Dukes have the same as sweets but do not range so high. Range of variation is the same in all. These data support the hypothesis that Dukes originate from hybridization between sweet and Morello cherries.

4. Stamen number is not closely associated with flowering or fruit ripening periods, juice colour, nor resistance to bacterial canker. Neither does variation within a variety indicate its potential fruit setting. Incompatibility groups I to VI tend to have lower numbers than the less frequent groups VII to XII. This may be associated with the *S* alleles.

5. Stamen number and variation is useful as an additional diagnostic character in cherries, and possibly other cultivated fruit species.

Thanks are due to Mr. M. B. Crane, F.R.S., for permission to use the J.I.H.I. cherry collection, to Dr. D. Lewis for use of his seedlings, and to Mr. A. G. Brown for helpful advice.

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Chromosome Number of *Laminaria digitata* Lamour

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With Plates 3 and 4 and three Figures in the Text

ABSTRACT

An investigation of the chromosome number of *Laminaria digitata* Lamour has revealed the diploid complement to be 16. Endomitotic chromosome multiplication has been observed. A similar investigation by Magne has shown the diploid number of chromosomes to be 26 in *Laminaria flexicaulis* Le Jolis and raises the taxonomical question whether these two names can be regarded as synonyms as at present used or covering distant species.

SPORES of *Laminaria digitata* collected at Dunbar, Scotland, were cultured during October to November 1952 in sea-water obtained from the Firth of Forth and enriched with sodium hydrogen phosphate and sodium nitrate; the solution was aerated daily.

Cover-slips placed on the bottom of the tanks were recovered at various periods of culture and examined after the following treatment: fixed in absolute alcohol-acetic acid (3 to 1) for 2 to 3 hours, hydrolysed in N.HCl for 10 minutes at 60° C., into Feulgen's reagent for 4 to 5 hours, washed in 45 per cent. acetic acid, dehydrated through alcohols of increasing concentrations to absolute, and mounted in Euparal. Slides were lightly warmed to increase differentiation.

The above treatment followed in principle the formula of Darlington and La Cour used for squashes with considerable modifications of time of the different stages.

The Feulgen reaction with chromosomes of *Laminaria digitata* varies in the following order of increasing intensity: female gametophyte, male gametophyte, sporophyte.

From the observations in this investigation, the diploid number of chromosomes of *Laminaria digitata* is $2n = 16$. This complement compares with $2n = 32$ found in *Macrocystis integrifolia* Bory (Walker, 1952) and $2n = 22$ found in *Laminaria cloustoni* (Walker, unpubl.).

The chromosomes as seen by the Feulgen reaction are spherical. (The threads sometimes seen connecting chromosomes when iron haemotoxylin is used are rarely seen after Feulgen reagent.)

After the nuclear wall of the male gametophyte cell has broken down, multiplication of the chromosomes occurs while dispersed in the cytoplasm (Text-fig. 1, E and F). The haploid complement of chromosomes contains

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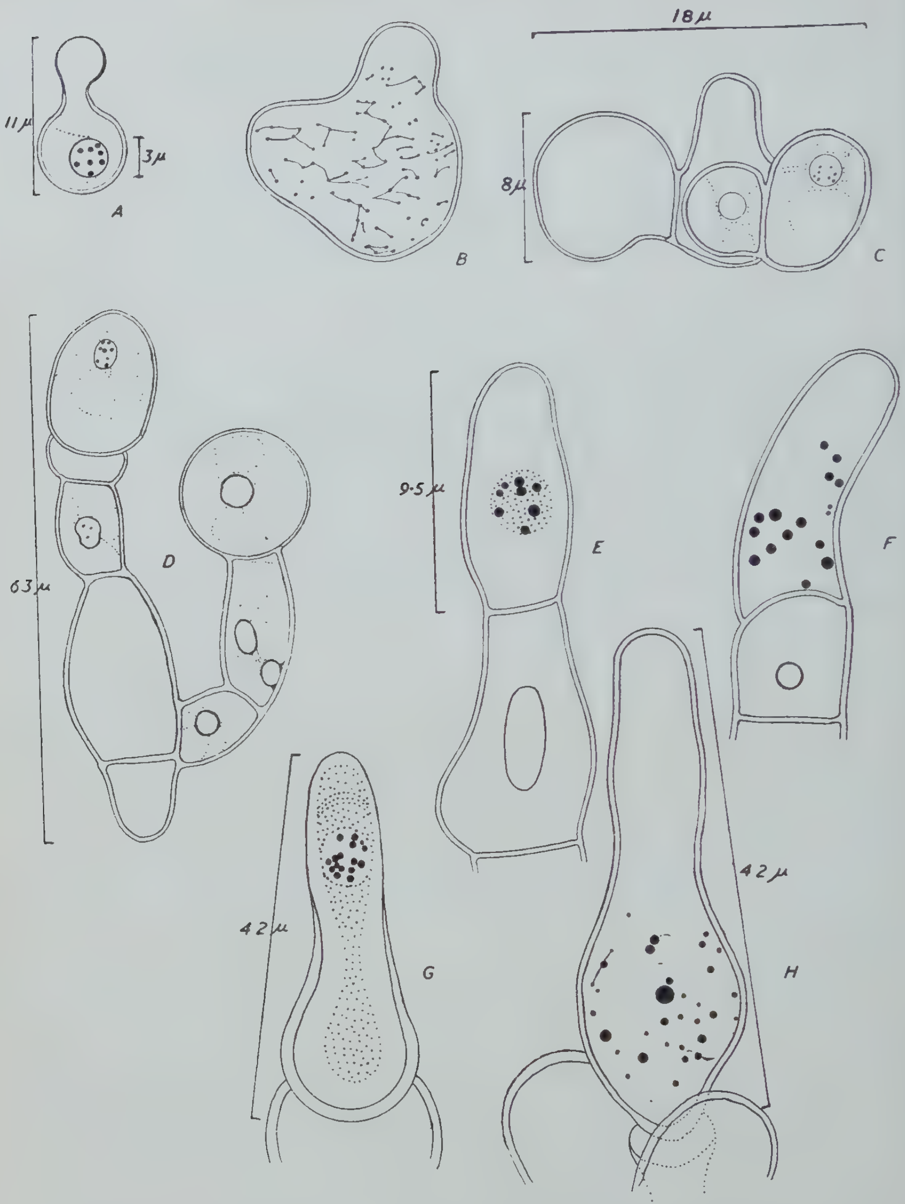

Text-fig. 1, A–H. *Laminaria digitata.* A. Developing spore, dumb-bell stage, empty spore case with first cell of gametophyte in formation; intact nucleus has passed out of spore; haploid set of chromosomes shown, $n = 8$. B. Female gametophyte in very early stage of cell division; nucleus dispersed in the cytoplasm as a precipitate of nucleic acids. C. Four-celled female gametophyte with intact nuclei one of which shows haploid set (8) of chromosomes. D. Nine-celled female gametophyte with intact nuclei one of which shows haploid set (8) of chromosomes. E. Terminal cell of male gametophyte, nuclear wall breaking down; haploid set (8) of chromosomes present. F. Terminal cell of male gametophyte, nucleus completely two chromosomes smaller than the rest; a similar relationship has been observed in the nucleus of the male gametophyte of *Laminaria cloustoni.*

The nucleus of the female gametophyte cell when in the resting stage shows distinctly against the cytoplasm which is colorimetrically unaffected by Feulgen's reagent (Pl. 3, A), but the female nucleus when dispersed in the cytoplasm is very granular, giving a pink colour to the whole cell contents (Text-fig. 1, B, and Pl. 3, B).

After fertilization of the cell containing the ovum, the diploid complement of chromosomes congregates (Text-fig. 1, G, and Pl. 3, C) and passes into an elongation of the oogonium which is later sealed off to form the embryo sporophyte. Feulgen reacting granules are seen in the cytoplasm at this stage along with well-defined chromosomes.

The nucleoplasm loses its colour reaction with Feulgens as the sporophyte nucleus enlarges prior to breaking up (Text-fig. 2, O). After the break-up of the nucleus the chromosomes become dispersed in the cytoplasm and may enlarge during this process; at this stage the chromosomes are most easily seen.

In the single-celled stage of the sporophyte, the chromosomes undergo endomitotic multiplication to polyploidy while dispersed in the cytoplasm (Text-fig. 2, J, and Pl. 3, C); wall formation follows later (Text-fig. 2, K, L, M, and Pl. 4, E, F).

Nuclei become proportionately smaller as cell division proceeds in the very young sporophyte, i.e. 40-celled sporophyte (Pl. 4, G):

Ratio of volumes of female gametophyte cell to nucleus	32:1
,, ,, ,, sporophyte cell to nucleus	32–36:1
,, ,, ,, female and sporophyte cells	2·3–2·6:1
,, ,, ,, ,, ,, ,, nuclei	2·7:1

Chromosomes also become smaller as cell and nuclear divisions proceed. The chromosomes vary in diameter, ranging from 1·2 to 0·3μ, a volume ratio of 64 to 1. The largest chromosomes of the sporophyte when seen dispersed in the cytoplasm are approximately $\frac{1}{16}$th of the volume of the resting nucleus of the female gametophyte.

From February 1947 to January 1948, 20 mature plants of *Laminaria digitata* were collected every month from Clachan Sound and Loch Melfort, south-west Scotland. The former locality is supplied with open sea-water while the latter is a sheltered sea loch. The mean weight and length of the plants were:

Clachan Sound (240 plants)	950 g.	240 cm.
Loch Melfort (240 plants)	550 g.	124 cm.

The plants from Clachan Sound were twice as long and twice as heavy as those

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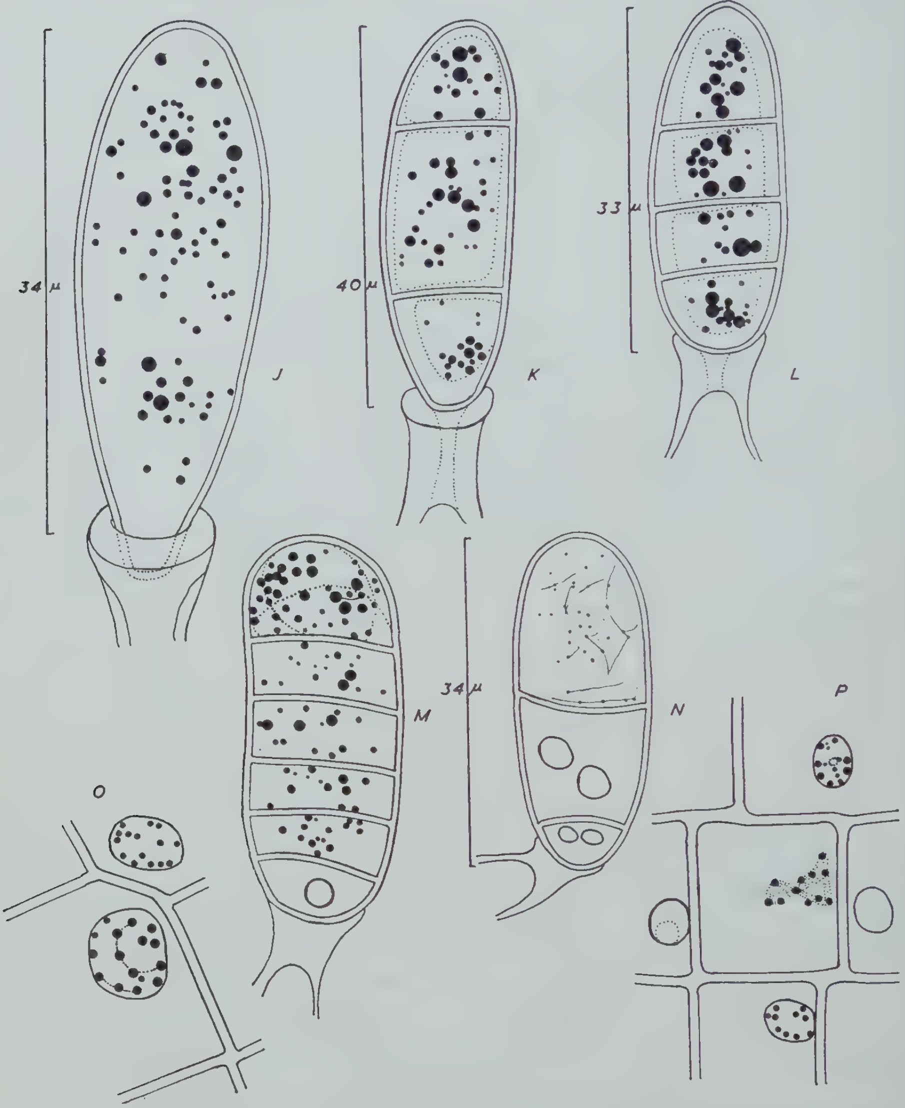
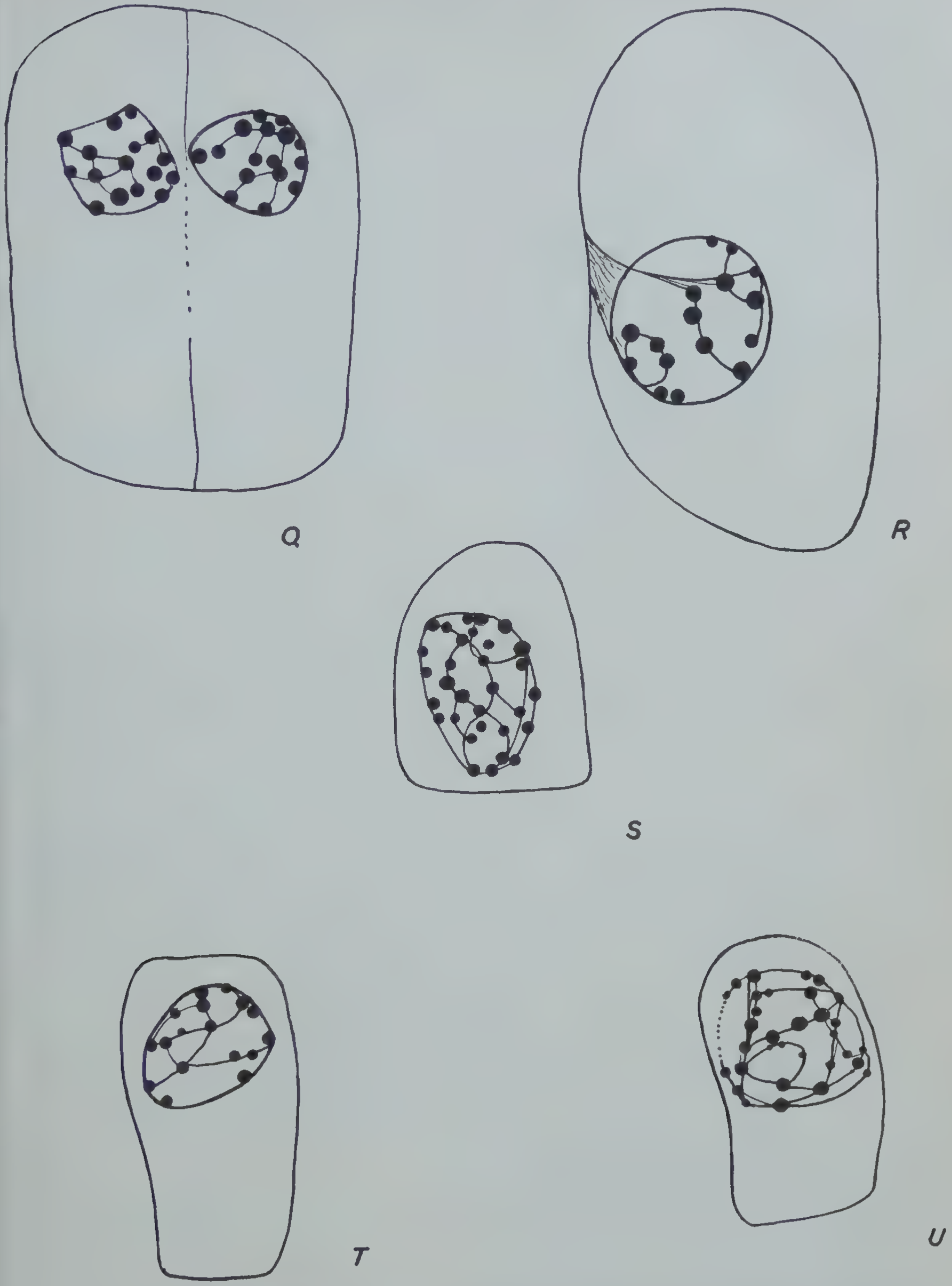
dispersed in cytoplasm; chromosomes have divided to produce 16 bodies, twice the haploid complement. G. Female cell elongating after fertilization preparatory to formation of a sporophyte; diploid set (16) of chromosomes present; nuclear wall ill defined; cytoplasm passing from oogonium to sporophyte. H. Single-celled sporophyte enlarging; diploid set of chromosomes have divided to give 32 daughter chromosomes. All fixed in alcohol-acid acetic passed through Feulgen's reagent after Darlington and La Cour modified Walker. Resolution $\frac{1}{12}$ O.I., N.A.1.25.



Text-fig. 2, j–p. *Laminaria digitata.* j. Single-celled sporophyte, chromosomes dispersed in cytoplasm undergoing endomitotic division. k. Sporophyte, with 4 diploid sets of chromosomes dispersed in the cytoplasm, forming transverse walls. l. Four-celled sporophyte with chromosomes dispersed in the cytoplasm. m. Eight-celled sporophyte; all nuclei except proximal one dispersed in the cytoplasm; distal cell chromosomes larger than other cells' chromosomes. n. Three-celled sporophyte; proximal and median cells with intact daughter nuclei, distal cell nucleus dispersing into the cytoplasm. o. Nuclei of a young multicellular sporophyte; one nucleus enlarging with colourless nucleoplasm and 16 chromosomes present, other nucleus coloured and 15 chromosomes present. p. Nuclei of young multicellular sporophyte with nuclei in various stages, mitosis does not occur simultaneously in all nuclei in the sporophyte. All fixed in alcohol-acetic acid and passed through Feulgen's reagent after Darlington and La Cour modified Walker. Resolution $\frac{1}{12}$ O.I., N.A.1.25.



Text-fig. 3, Q–U. *Laminaria digitata* epidermal cell nuclei. Q. Telophase, 16 chromosomes in each daughter nucleus. R. Late prophase, 16 chromosomes present. S. Metaphase, 31 chromosomes present. T. Prophase, 17 chromosomes present. U. Metaphase, 32 chromosomes present. Q, R, S from a plant collected from Atlantic Bridge, Clachan Sound; T, U from a plant collected from Loch Melfort. All fixed in chrom-acetic acid and stained with Delafield's iron haemotoxylin. Resolution $\frac{1}{12}$ O.I., N.A.1.25.

from Loch Melfort. Polyploidy was suspected and a cytological investigation was therefore undertaken of the nuclei of the epidermal cells of the laminae of plants from both localities.

For the sake of impartiality, the nuclei were selected and drawn by Miss L. M. P. Whiteside; a selection is shown in Text-fig. 3. Counting of the chromosomes was found to be $2n = 16$ for the plants from both localities even with so much difference in their sizes. This earlier investigation confirms the diploid number of 16 found in material cultured from spores.

How the diploid complements of chromosomes are sorted out when wall formation follows endomitotic chromosome multiplication is a problem resulting from this study, but they are for 4, 8, and 16 nuclei have been seen in single-celled sporophytes.

Since this paper was completed Magne (1953) has sent me a copy of his paper in which he states that *Laminaria flexicaulis* Le Jolis has a haploid complement of 13 chromosomes as seen by Feulgen treatment. The question is now for the taxonomists to decide whether *Laminaria digitata* Lamour and *Laminaria flexicaulis* Le Jolis are the same species. In this connexion, it is worth reading the original paper by Le Jolis (1854) where he sets out to justify, at great length, the separation of *Laminaria digitata* of Lamouroux into two distinct species which he named *Laminaria flexicaulis* and *Laminaria cloustoni*. The plant known in Norway as *L. flexicaulis* is the one named *L. digitata*, var. *stenophylla* Harv. in Britain: this plant has $2n=26$ chromosomes.

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EXPLANATION OF PLATES

Illustrating F. T. Walker's paper on 'Chromosome Number of *Laminaria digitata* Lamour'.

PLATE 3

A. Female gametophyte, one cell with intact resting nucleus and another with two daughter nuclei and dividing wall in process of formation.

B. Female gametophyte with nucleus dispersed in the cytoplasm of each cell.

C. Elongation of oogonium to form sporophyte, dispersed chromosomes passing up.

D. Sporophyte with chromosomes undergoing endomitotic multiplication.

PLATE 4

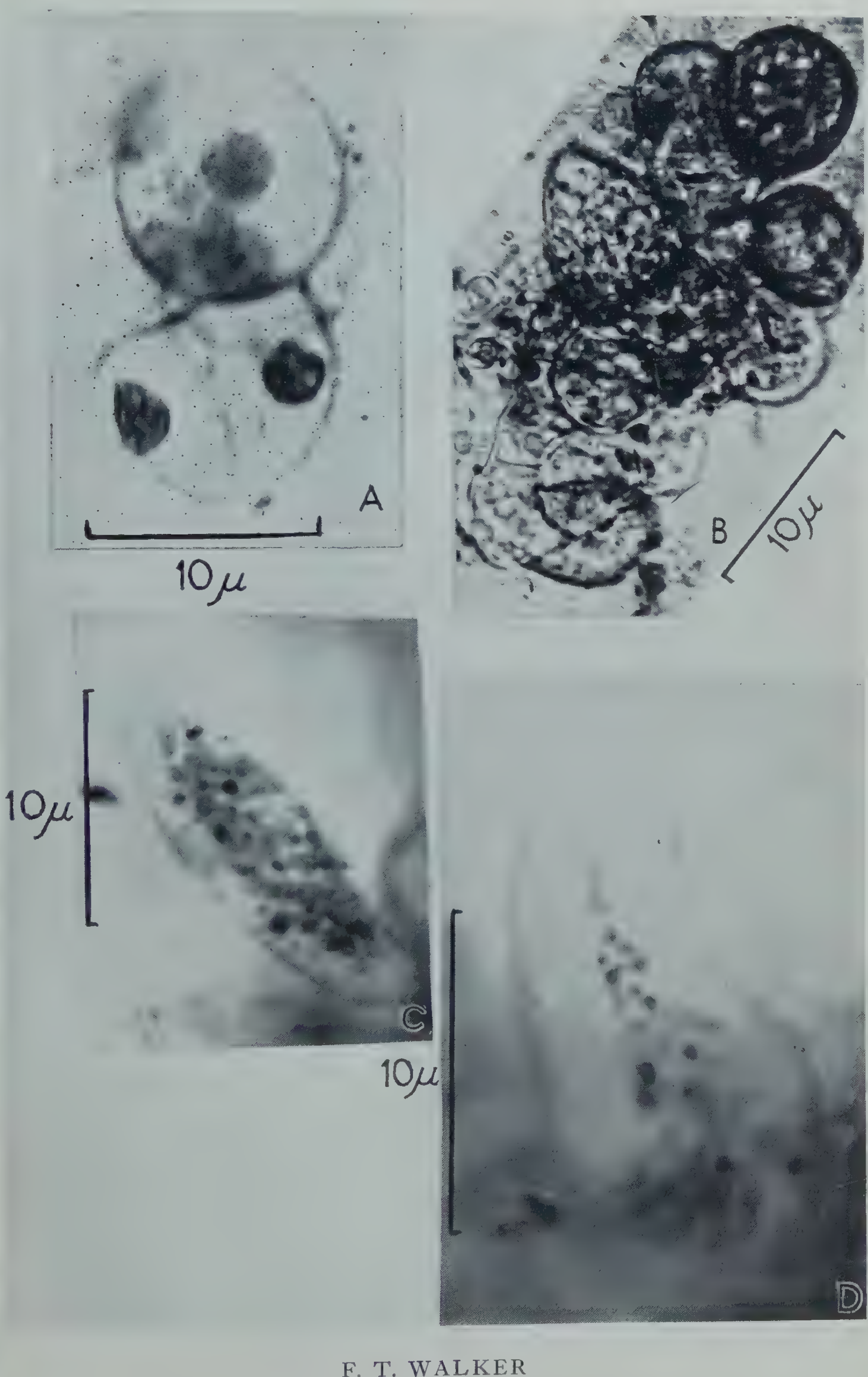
E. Four-celled sporophyte with chromosomes dispersed in the cytoplasm.

F. Eight-celled sporophyte with chromosomes dispersed in the cytoplasm.

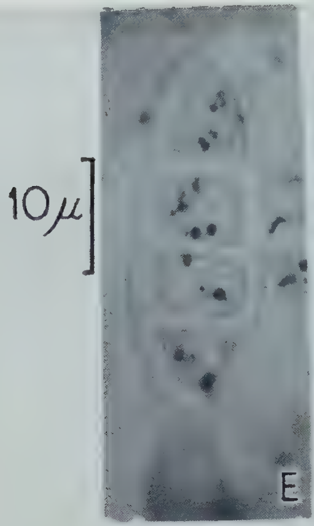
G. Forty-celled sporophyte, nuclei in prophase.

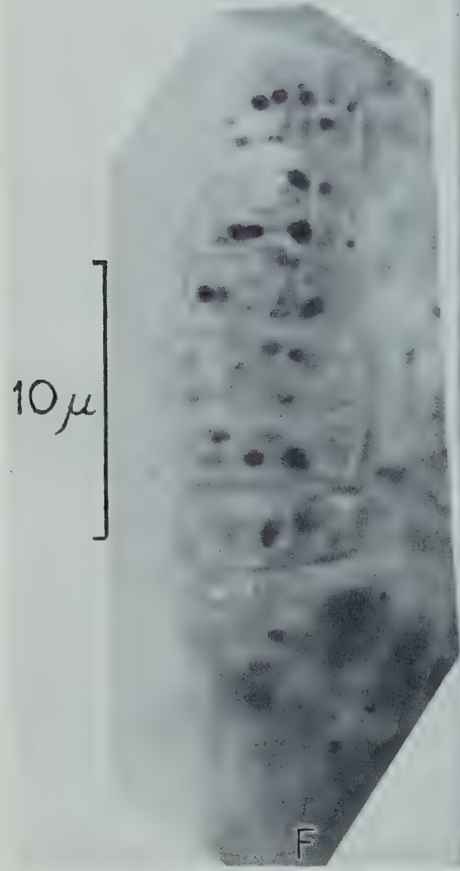
All fixed in alcohol-acetic acid and treated with Feulgen's reagent, Darlington and La Cour (modified Walker) formula for squashes.

The black size-lines are 10μ long.



F. T. WALKER





F. T. WALKER

Vegetative Proliferation of Floral Spikelets in British Grasses

BY

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ABSTRACT

Proliferation of spikelets in grasses other than those races which are habitually viviparous is reviewed, and the factors inducing proliferation experimentally in *Cynosurus cristatus* and other species are discussed. It is concluded that the evidence favours the hypothesis that in the viviparous races a considerably greater minimal concentration of the putative flowering hormone is required for flower induction than for culm initiation, whereas in the seminiferous races this difference is not so great. In the viviparous races, the threshold for flower initiation is rarely exceeded so that perfect flowers appear only occasionally, whilst in the normally seminiferous races, the conditions arise only rarely where an amount of hormone is produced sufficient to initiate culms but insufficient to promote flowering.

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1. INTRODUCTION

AT times grasses may show vegetative proliferation of their flower spikelets, that is, the production of leaves distally upon the spikelet axis. The leafy portion of the proliferated spikelet may then become detached and serve as a diaspore for the plant's propagation. Certain grasses regularly reproduce by this vegetative means and these may be distinguished as the 'viviparous races'.

In the viviparous races proliferation of spikelets is a hereditary character scarcely modified by environment.

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The application of the term 'vivipary' to proliferation of spikelets in general has led to confusion. In the first paper of this series the confused terminology, the constancy of vegetative development of the spikelets in the viviparous races, and the taxonomic position of these forms were discussed.

In the second paper the natural geographical and ecological distribution of the viviparous races in Britain was described. The viviparous races survive in areas and habitats where moist conditions prevail. However, since transplants of these races are constant in their proliferation, humidity is not the cause of the phenomenon. Humidity is essential for the establishment of the leafy plantlets of some viviparous forms.

In this paper it is intended to examine the circumstances of spikelet proliferation in plants, which are not members of the viviparous races. It is characteristic of the majority of cases to be reviewed that the plants concerned normally produce flower-bearing culms, but occasionally the spikelets in whole or part of an inflorescence are proliferated. The proliferations may be used as cuttings and reared into adult plants, but they do not inherit any tendency to proliferate in normal conditions.

Following the discussion of ephemeral proliferation an hypothesis to explain the physiological basis of proliferation is advanced. This hypothesis is extended to cover the viviparous grass races also.

2. Occurrence of Ephemeral Proliferation

In Table I is a list of species in which proliferation has been recorded. The so-called viviparous races, in which the feature is primarily hereditary, are omitted and records which appear confused are also excluded.

TABLE I

Records of Proliferated Spikelets in Some British Species

Species.	Observer and notes.						
Agropyron repens Beauv.							Wy[2]
Alopecurus pratensis L.			J[3]				
Arrhenatherum avenaceum Beauv.	A[2]						
Cynosurus cristatus L.	A[2]		J[3]		Th[3]	Wa[2, 4]	Wy[1, 2, 4]
Dactylis glomerata L.	A[2]			S	Ti[1]	Wa[2]	Wy[2]
Deschampsia caespitosa Beauv.			J[1]	L[1]	Ny[1]		
Festuca rubra L.			J[3]				Wy[2]
Lolium perenne L.	A[2]	C[3]	J[3]	M[1]		Wa[2]	
Phleum pratense L.	A[2]	E	J[3]	S[3]	Ni		
Poa trivialis L.			J[1]				

[1] = In greenhouse or experimental conditions.
[2] = Date given as last week of August or later.
[3] = Proliferation stated to be more frequent in autumn.
[4] = In some specimens of *C. cristatus* both fertile and sterile spikelets are proliferated.

A	Arber (1934).	C	Cooper (1951).	E	Evans (1927).
J	Jenkin (1922).	L	Lawrence (1945).	M	Margadant (see text).
Ni	Nielson (1941).	Ny	Nygren (1949).	S	Schuster (1910).
Th	Thoenes (1929).	Ti	Tincker (1925).	Wa	Wakker (1891).
		Wy	Wycherley (1952) (and below).		

A. repens (1/9/52), *C. cristatus* (19/10/49), *D. glomerata* (9/10/50 & 16/11/52), and *F. rubra* (13/9/50).

Obviously some species are more prone to this abnormality than others. The majority of cases of transient proliferation occur in the autumn. Arber (1934) and Schuster (1910) comment that proliferation is not purely evanescent in *Phleum pratense* L. Certain strains respond more readily to autumn conditions by proliferation of their spikelets. These may be regarded as an approach towards a viviparous race.

Because ephemeral proliferation is more common in autumn and the arctic-alpine viviparous races have a predilection for humid habitats, it might be supposed that moisture was a causal agent. However, humidity for those viviparous races is necessary for the establishment of plantlets, but not for the induction of the phenomenon. Moreover, *Poa bulbosa* L. var. *vivipara* Koel. is constant in proliferation in its natural arid habitat. Its success in dry conditions is attributed to the bulbils' succulence.

Proliferation commonly occurs in autumn when the natural day-length is decreasing. The effect of this condition is considered in the next section.

3. Proliferation Experimentally induced

Photoperiodic experiments. The short photoperiods of autumn may cause the vegetative development of inflorescences induced in long-day plants. Thus the 'tussock type' of *Dactylis glomerata* L. flowered in natural summer long days, but bore proliferated inflorescences in 12-hour photoperiods, while plants of the same clone were wholly vegetative in 6-hour and 9-hour days (Tincker, 1925). Nygren (1949) caused proliferation of spikelets in normal seminiferous *Deschampsia caespitosa* (L.) Beauv. by short-day treatment.

Flowering is induced in *Cynosurus cristatus* L. by long days. Earlier (Wycherley, 1952) short-day treatment was applied to *C. cristatus* plants which had made some progress towards inflorescence initiation. This treatment resulted in strong proliferation of the sterile spikelets and sometimes of the fertile spikelets too.

Some clonal material of this species was available and it was decided to repeat this experiment. It was hoped that the use of clonal material would result in a continuous series from vegetative through proliferated inflorescences to normal flower-bearing plants with increasing periods of continuous light treatment before transfer to short days.

The plants were overwintered in natural open conditions until February 20, and afterwards they received various periods of continuous light in a warm greenhouse (approx. 20° C.). The continuous illumination in warmth began at 1600 hours and continued for multiples of 24 hours. After this treatment the plants were placed in the short-day section of the greenhouse (darkened from 1630 hours until 0830 hours). Controls remained in continuous illumination and in short days throughout. Representatives of each clone were dissected after 0, 5, 10, and 15 days' continuous illumination. No great variation in the stage of differentiation reached was observed between the three clones used.

The treatments were given to duplicate pairs of plants, and the extent of the experiment was limited by the amount of material available. The results are in Table II.

Although the three series are clones the plants of each series do not show a perfect transition from vegetative plants to flowering ones. Possibly the tillers at the time of founding the clones were not all of the same physiological age, and also, although the temperature variations from day to day and between the two sections were not large, they may have been sufficient to upset the balance of various processes.

TABLE II

Behaviour of Clones of Cynosurus cristatus

Days of continuous light before transfer to short days . .	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	∞
State of growing-point . .	V	—	—	—	—	V	—	—	—	—	DR	—	—	—	—	IB	—	—
Clone *P*	V	V	V	V	V	V	V	P	P	P	—	—	—	—	—	—	—	F
	V	V	V	V	N	V	V	P	F	*p*	—	—	—	—	—	—	—	F
Clone *Q*	V	V	V	V	N	N	V	V	F	F	F	F	F	F	F	—	—	F
	V	V	V	V	P	D	F	P	P	P	F	F	*p*	F	*p*	—	—	F
Clone *R*	V	—	V	—	V	—	N	—	D	—	N	—	N	—	N	—	N	F
	V	—	V	—	V	—	V	—	V	—	V	—	V	—	V	—	N	F

D = Dead.
DR = Double ridges.
F = Normal flowering.
IB = Initiated panicle branches.
P = Proliferation of fertile and sterile spikelets.
p = Proliferation of sterile spikelets.
N = Inflorescence never emerged.
V = Vegetative.

'Double ridges' are the first signs of inflorescence initiation; 'panicle branches' is a later stage in which the upper members of the double structures are growing out and differentiating further primordia.

In clone *R* inflorescences induced by continuous light are unable to emerge in short days. Indeed, short photoperiods have a markedly depressing action on this clone.

The results for clones *P* and *Q* indicate two conclusions. Firstly, an inflorescence may develop in short photoperiods although at the time of transfer from flower-inducing conditions no visible signs of initiation could be detected. Secondly, proliferation of spikelets occurs when the period of induction is minimal for culm initiation.

Similar conclusions were reached in earlier experiments (Wycherley, 1952). However, the observations reported here draw attention to a further point.

After 10 days' continuous illumination the growing points of clone *Q* plants had differentiated 'double ridges'. The uppermost ridges of these twin structures are panicle branch primordia. Most of the clone *Q* plants which received 10 or more days' continuous light bore later in short days normal inflorescences with fertile florets and unproliferated spikelets (panicle emergence was usually about 6 weeks after transfer). But after lesser periods of continuous illumination most plants were vegetative or bore strongly proliferated inflorescences.

Thus in this experiment about 10 days' continuous illumination may be regarded as a critical treatment. There is a tendency towards vegetative growth after less than 10 days' illumination, but after more than 10 days the trend is for full floral development. But at the conclusion of the tenth day of continuous light only an early stage of inflorescence differentiation has been reached. Thus the primordia, which differentiated as leaves or bracts according to treatment, were not yet initiated. Therefore the primordia are affected not only by the photoperiod at the time of their initiation and development, but also by the sum of the plant's history.

Theoretical discussion is deferred until other cases have been reviewed.

So far plants that flower after long-day induction have been discussed. Galinat and Naylor (1951) found that the maize strain, C31 id, was very prone to proliferation of the male inflorescence. This plant is of the short-day class; photoperiods of 10½–11½ hours per day produced normal staminate flowers, in 11½–12½ hours the flowers were sterile, and in photoperiods of up to 15 hours proliferations were obtained. These proliferations could be cut off and struck. In longer photoperiods this maize was vegetative. Clearly again marginal photoperiodic treatment results in proliferation.

Temperature treatment. Margadant transferred a series of *Lolium perenne* L. plants from greenhouse condition to the open, so that they received successively less natural winter (vernalizing) treatment before experiencing normal spring and summer conditions. In a group transferred late, most of the plants were vegetative, but one bore a vegetatively proliferated culm, which was given to the present author. Insufficient vernalization may therefore be another cause of proliferation. Cases of proliferation in greenhouses (Jenkin, 1922) might be attributed to insufficient cold.

Deschampsia caespitosa var. *genuina* plants were collected by Lawrence (1945) from Lappland (68° N.), Finland, Sweden, and three stations in California at approximately 30, 1,400, and 3,050 metres elevation. At each of the Californian stations specimens of each group were grown. Plants from Lappland bore proliferated inflorescences at all three Californian stations. Those of Finnish and Swedish origin produced proliferations at the two lower stations. Transplantation of local plants from the highest station to the lowest resulted in moderate proliferation of spikelets. Change of day-length alone may have been responsible for proliferations in the plants of Scandinavian origin; but, especially in the last-mentioned case, insufficiently cool winter conditions and hence lack of complete vernalization is an equally important possibility.

A rather different state of affairs was obtained by Kostoff (1940) with rye hybrids (8th generation backcross to *Secale cereale* L. of *S. cereale* × *S. montanum* Guss). In summer the perennial forms overwintered at 0–10° C. gave proliferated spikelets (which could be used for propagation as cuttings). Overwintering at 15° C., however, produced only normal flowers. Short-day induction replaces cold vernalization in some winter ryes; thus it is not easy to postulate with certainty the possible physiological interactions.

Inflorescence induction in the viviparous races. Before considering hypotheses to explain proliferation, the conditions for inflorescence induction in the viviparous races will be discussed.

Cold vernalization or cold in conjunction with short days would appear to be a necessary pretreatment for inflorescence induction in the British viviparous grasses. Plants of seminiferous *Deschampsia caespitosa*, *Poa alpina*, and *Festuca ovina*, and of viviparous Deschampsiae, Poae, and *Festuca vivipara*, were overwintered in the open, in a warm greenhouse and in a continuously illuminated greenhouse. In the following summer only those overwintered in the open bore culms.

Plants gathered from the Cairngorms (elevation 1,200 m.) in September (when at this altitude it was already freezing frequently during the nights) produced culms in the continuously illuminated greenhouse, but did not bear inflorescences again without wintering outside.

4. General Hypothesis

Theoretical speculations on proliferations in Cynosurus cristatus. Inflorescence initiation and flowering in *C. cristatus* are induced by long days. Presumably long days produce a substance or condition whose concentration or intensity is proportional (but not necessarily directly proportional) to the period of induction.

For simplicity of discussion the final flowering stimulus is assumed to be a substance, say *F*. It is presumed that the operative concentration of *F* is that at the time of initiation and differentiation of an organ. To interpret proliferation in *C. cristatus* there are two main possibilities, which may both be true.

(i) As inflorescence development proceeds the concentration of *F* is reduced either by use during initiation or destruction during unfavourable photoperiods. Thus even if the minimal concentrations for culm and floret initiation are the same, full floret development may fail.

(ii) Alternatively the critical concentrations of *F* necessary to induce culms may be lower than that required to induce florets, which may be lower than that needed to inhibit vegetative growth of the sterile spikelets. Thus the longer the period in conditions favourable to induction, the greater the degree of flowering and suppression of vegetative growth.

The main point of (i) is loss of *F* by use or destruction, while in (ii) attention is drawn to the possibility of higher critical levels as differentiation of the inflorescence proceeds.

Ephemeral proliferation. A quantitative hypothesis of proliferation might postulate that the concentration of flower-inducing substance *F* is proportional to the intensity and duration of induction treatment, and that, if (relative to the critical level necessary for flower induction) there is a deficit of *F* after culm initiation, vegetative proliferation of spikelets will result.

In the species for which proliferation is an ephemeral condition, probably the concentrations of *F* necessary for culm and floret initiation are the same or nearly so. Only rarely will insufficient *F* be available to induce florets after culm initiation. This deficit may be due to insufficient vernalizing pretreatment or incomplete photoperiodic induction.

In nature most of the ephemeral proliferations in long-day grasses are of serotinous culms. Whether or not flower-inducing hormone is used up in flowering, it is probably lost in the tillers that flower because they are determinate structures. Insufficient may be present in the late culms to initiate florets.

Proliferation in the viviparous races. The culms of the viviparous races normally merge in midsummer; thus there are three possible explanations why floret initiation fails.

(*a*) Sufficient vernalization is rare.
(*b*) The drain on flowering hormone during culm initiation is very great.
(*c*) A greater minimal concentration is required for flower induction than for culm initiation.

The constancy of proliferation in transplants of the viviparous races suggests that some fundamental difference is responsible. The degree of vernalization depends on winter conditions and is highly unlikely to reach a fine marginal level every year. Therefore (*a*) is dismissed.

Considering possibility (*b*). Throughout the time of culm formation the viviparous plants are in conditions favourable to flower induction. Therefore after some vegetative growth florets might be expected distally in the spikelets, but proliferated spikelets of these races bear florets proximally. Thus (*b*) is also rejected and (*c*) is accepted as the most reasonable assumption.

Therefore the regular proliferation of spikelets in the viviparous races may be due to the critical level for floret induction being so much higher than that necessary for culm formation, that this higher level is normally never reached.

There are, of course, difficulties. The viviparous fescues occasionally produce fully flower-bearing panicles (Jenkin, 1922; Turesson, 1926–7). These may be caused by the concentration of F reaching the higher level on rare occasions. It was noted in the first paper of this series that in viviparous Deschampsiae exposed to conditions that promoted shooting, the treatment also stimulated increased flower-bearing.

If this hypothesis is true, farther north, where the winters are colder and the summer day-lengths longer, the degree of proliferation might be expected to be less. There is no detailed knowledge on this subject, although Flovik (1938) reported viviparous grasses from Spitzbergen. However, if the cold is very deep there will be no metabolic activity and vernalization will fail to be very intense. Moreover, northern conditions may have selected new strains, which are proliferated in the extreme environment. Lawrence, as already mentioned, has demonstrated proliferation in seminiferous forms moved south.

Another difficulty is to apply this hypothesis in its simplest form to the semiviviparous forms, whose spikelets are constantly partially proliferated.

The lower bracts have florets in their axils, but the upper part of the axis bears foliage leaves. However, an indication may be obtained by considering ephemeral proliferation of grasses again.

In many of the proliferated specimens examined by the present author the proliferation was strongest on the lower spikelets in the spike or panicle. This appears to be true of the plants illustrated by Evans (1927). In many grasses (see, for example, Sharman, 1947) the differentiation of spikelets is in basipetal succession, but the differentiation of florets in a spikelet proceeds acropetally. If the concentration of F is falling during the period when spikelets are differentiating, the first formed will be floret-bearing, but the latter spikelets will be vegetatively proliferated. Such a condition may regularly arise in the semiviviparous plants, flower-inducing substance failing in a spikelet after the initiation of the basal florets. This explanation, which is a combination of alternatives (*b*) and (*c*), is not wholly satisfactory, although it may serve as an hypothesis.

Even if this application is not justified, the hypothesis is useful in conjunction with a study of the order in which organs are differentiated, wherever there is variation within one inflorescence which it is desired to explain on the basis of only one postulated hormone or assumed influence.

From the viewpoint of the acceptance of this hypothesis as a general guide the origin of the viviparous races might be accounted for, either by Nygren's (1949) theory of the hybridization of forms with different life-rhythms or by mutation. The latter is easier for the present author to visualize as the cause of a stable arrangement.

5. SUMMARY

1. Proliferation of spikelets in grasses other than the viviparous races is reviewed.

2. Experimental induction of proliferation in *Cynosurus cristatus* is considered in detail. At the conclusion of a critical period of continuous illumination the primordia of the affected organs were not yet initiated in plants transferred to short photoperiods.

3. It is suggested that the concentration of a flower-inducing hormone, F (or the intensity of some condition), at the time of initiation and differentiation of the inflorescence organs is responsible for the degree of floral or vegetative development shown.

4. In the ordinary seminiferous grasses the critical concentrations for the induction of the culm and florets are supposed to be the same or the latter only slightly greater. Thus, only very rarely will enough hormone be present to initiate a culm but insufficient to induce flowering. Attention is drawn to the order of differentiation in the panicle, since some floret-bearing spikelets may be differentiated while the concentration is greater than when proliferated spikelets are developed.

5. This hypothesis is applied to the viviparous races, and it is postulated

that the difference in minimal concentrations of F for culm and floret induction differ greatly, the latter being higher in these races. Normally floret induction is never achieved.

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